THE PHILIPPINE

JOURNAL OF SCIENCE

VOL. I NOVEMBER, 1906. No. 9.

THE CULTIVATION AND PATHOGENESIS OF AMŒBÆ.

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CONTENTS.

I. INTRODUCTION.

II. BIOLOGY OF AMŒBÆ.
1. Review of recent literature.
2. Methods of reproduction and life cycle.
3. Morphology, classification, etc.
(*a*) Amœbæ as a whole.
(*b*) Ectoplasm.
(*c*) Endoplasm.
(*d*) Nucleus.

III. CULTIVATION.
1. Methods and material.
2. Pure cultures.

IV. SYMBIOSES AND SYMBIOTIC ORGANISMS.
1. Extraneous or saprophytic.
2. Cultural.
3. Intestinal.
(*a*) Saprophytic.
(*b*) Parasitic.
4. The higher animal tissue symbioses or true parasitism.
5. The alterations in symbioses.
(*a*) Increasing processes, parasitism, virulence.
(*b*) Decreasing processes, saprophytism, avirulence.

V. PATHOGENESIS OF AMŒBÆ.
1. Critical review of some recent literature.
(*a*) Amœbæ in healthy persons.
(*b*) Pathogenic and non-pathogenic amœbæ.

V. PATHOGENESIS OF AMŒBÆ—Continued.

2. Original work.

(*a*) Experimental amœbic colitis.

(*b*) Experimental amœbic abscesses of liver.

(*c*) Experimental amœbic abscess of the ŏmentum, spleen, lung, ĕtc.

(*d*) Experimental amœbic septicæmia.

(*e*) Immunity.

(*f*) Amœbic lesions (how produced).

VI. SUMMARY.

I. INTRODUCTION.

Since our first publication in 1904, on the cultivation and etiologic significance of amœbæ,[1] a number of articles dealing with the subject have appeared which in the main have been confirmatory of our results.

Our own work has been continued and, in addition to confirming the observations given in the first paper, enough new facts have been established to make further publication at this time desirable. As we have absolute control of the large clinical and pathological material of St. Paul's Hospital and free access to that of Bilibid Prison Hospital as well as to numerous other less important sources, our opportunities have been all that could be desired.

In studying the more purely biological problems connected with this subject, we have felt the lack of advice and association with a trained zoölogist, and for that reason have avoided discussing much that is interesting and definite in the study of cultures of amœbæ. However, there are a number of points in this connection which have clearly been established, and, notwithstanding the fact that the results differ in certain instances from the expressed views of some eminent zoölogists, they are given with full conviction of their accuracy, together with the experimental proof.

The most important question dealt with in this paper (and it seems to us that this is the keystone to the whole problem of amœbic infection) is the property, possessed by apparently saprophytic amœbæ from the outside world, by virtue of which the amœbæ become so modified by successive stages and changes in their environment or by symbioses that they become true parasites for monkeys, guinea pigs, and human beings.

Our experiments demostrate beyond doubt that this is the property of many amœbæ and this fact makes clear much which has formerly been confused about the pathogenesis of amœbæ.

As much of this work is so closely connected with the observations and conclusions given in our original paper, it is suggested that the reader consider that article in connection with this one.

[1] Amebas: Their Cultivation and Etiologic Significance, by W. E. Musgrave, M. D., and Moses T. Clegg, *Publications of the Bureau of Government Laboratories, Biological Laboratory*, Manila (1904), No. 18.

II. BIOLOGY OF AMŒBÆ.

In addition to the strictly biological problems, such as the determination of species, life cycle, etc., there are others more nearly bordering upon pathology, the accurate determination of which is vital to an understanding of the disease-producing properties of the parasites. This, for example, is shown in divisions of species into pathogenic and non-pathogenic ones. We do not like to take issue with the zoölogist upon the establishment of species, but when he carries his work as far as the determination of the pathogenicity of individual species, we feel justified in entering the discussion and in so doing it may be necessary, in meeting the issues involved, to use some criticism of a destructive character upon questions which are purely zoölogical, without offering a remedy. To this extent only will we invade the field of the zoölogist, with the exception of considering views which bear directly upon the medical aspect of the subject.

Many discussions of life cycles, classifications, and methods of reproduction of amœbæ, of interest to medical men, have been published from time to time, but these have largely fallen into disuse from lack of confirmation and therefore need not be entered into here.

REVIEW OF RECENT LITERATURE.

In 1903, the late Prof. F. Schaudinn[2] contributed valuable information on this subject, which at once attracted much attention. Many of his observations have repeatedly been confirmed, while other of his statements have been elaborated by subsequent writers. Schaudinn established a new genus, *Entamœba,* to include those amœbæ found in the human intestine, and in this new genus he described two species *E. coli* and *E. histolytica;* the former the so-called harmless commensal and the latter a true dysentery producer.

Briefly his observations were as follows:

The genus *Entamœba* includes the amœbæ which inhabit the intestine of man. Two species are described, one pathogenic and the other non-pathogenic; they may both at times be found in the same *diseased* intestine, but obviously not together in a normal bowel.

E. coli (the type species) is considered to be harmless and perhaps to be the one reported by Lösch,[3] but first accurately described by Casagrandi and Barbagallo. *E. histolytica* is the pathogenic species and is identical with Jurgen's cat amœba.

The chief characteristics of these two species are as follows:

E. coli, when at rest, shows no sharp distinction between ectoplasm and endoplasm, but this differentiation occurs to a moderate degree in the pseudopodia of the moving parasite.

The nucleus is centrally placed, vesicular (during the vegetative stage), and has a distinct, compact, nuclear membrane. In the center of the nucleus is a nucleolus, which is formed of plastin and chromatin. Other chromatin is also

[2] *Arb. a. d. k. Gsndhtsamte,* Berl. (1903), 19, 547–576.

[3] Schaudinn states that Lösch's description is so incomplete that he is unable to say whether Lösch had first encountered *E. coli* or *E. histolytica;* however, he is inclined to believe from Lösch's description that *E. coli* was the species described.

distributed in the nuclear cavity as an achromatic network. Multiplication takes place by fission and spore formation. In reproduction by spore formation, the chromatic substance of the nucleus divides into eight parts and, after solution of the nuclear membrane, these become arranged in the protoplasm of the amœbæ as daughter nuclei, and from these nuclei young amœbæ eventually always result.

This reproduction is brought about in a complex manner, somewhat as follows: During the encystment of the amœbæ, fructification occurs. The nucleus divides mitotically, and from the resulting two nuclei two pairs of daughter nuclei are formed in such a manner that each pair has an element from each original nucleus which has been produced by mitotic division. After wandering in the protoplasm for a certain time, these two pairs of daughter nuclei coalesce, so that we again have produced a cell with two nuclei. After a time these nuclei again part by primitive mitosis, giving rise to four nuclei and this process is finally once more repeated, giving an amœba with eight nuclear cysts. About each of these new cysts protoplasm collects, and from them eight young amœbæ develop in the next host.

E. histolytica differs sharply from *E. coli* in having a distinctly formed ectoplasm, which is very refractile and tenaceous. This latter property of the ectoplasm of this amœbæ enables it to penetrate between the epithelial cells lining the mucosa of the colon.

The nucleus of *E. histolytica* is poor in chromatin and it therefore is difficult to recognize; it changes its shape easily and is always excentrically located.

This amœba multiples by fission and a kind of budding or sporogony, the nucleus dividing amitotically. Under unfavorable conditions spores are formed. The nucleus expels some chromatin into the endoplasm, degenerates, and may then be seen to be extruded from the parasite. As the little masses of chromatin approach the periphery, the ectoplasm becomes bulged out and finally the particles of chromatin, with little masses of protoplasm measuring from 3 to 7 μ in diameter, are cut off and become separated from the remainder of the amœba, which now dies. These masses contain a concentric, thread-like structure, secrete an opaque membrane and the further processes of development finally become invisible. These bodies constitute the new infecting agent.

The paper of Schaudinn attracted a great deal of attention and some more recent observers have confirmed his results; others have accepted them as established and a few, in addition to confirming his principal observations, have elaborated other characteristics which distinguish between the species. Among the latter, Charles F. Craig's work[4] may be noticed.

Craig's conclusions, broadly speaking, confirm those of Schaudinn, but he proposes *E. dysenteriæ* as the name of the pathogenic species. His descriptions differ from those of Schaudinn somewhat in minor details and he adds a number of other distinguishing points which are intended to render the determination of species more practicable from a simple microscopical examination of the fæces.

Craig's summary of the differences between these species is as follows:

FRESH PREPARATION.—*Size.*—*E. dysenteriæ* usually considerably larger than *E. coli.*

Color.—*E. dysenteriæ* considerably lighter in color and much more refractive than *E. coli.*

[4] *Am. Med.* (1905), 9, 854, 897, 973.

Protoplasm.—E. dysenteriæ: Shows well-marked distinction between ecto- and endoplasm. This is more marked in the moving organism. *E. coli:* Never a sharp distinction between ecto- and endoplasm, even when the organism is moving. *E. dysenteriæ:* Ectoplasm much more refractive than endoplasm; the opposite is true in *E. coli.* *E. dysenteriæ:* Ectoplasm finely granular; *E. coli* structureless. *E. dysenteriæ:* Usually has one or more vacuoles; *E. coli* rarely shows a vacuole.

Nucleus.—E. dysenteriæ: Nucleus usually invisible and when present contains but little chromatin and has no marked nuclear membrane. *E. coli:* Nucleus almost always visible, shows a well-marked, rather thick, nuclear membrane which is very refractive to light. Nucleoli and a large amount of chromatin present.

Contained bodies.—E. dysenteriæ often contains large numbers of red blood corpuscles, crystals, bacteria, etc. *E. coli* rarely contains red blood corpuscles (never more than two or three); crystals, bacteria, etc., also diminished.

Motility.—E. dysenteriæ: Motility more marked and definite. *E. coli:* Motility very sluggish often difficult to distinguish. (This point alone is sufficient to distinguish between these organisms in fresh specimens.)

STAINED SPECIMENS.—*Protoplasm.—E. dysenteriæ:* Ectoplasm takes much deeper stain than endoplasm. *E. coli:* Endoplasm takes much deeper stain than ectoplasm. (This point alone is diagnostic.)

Nucleus.—E. dysenteriæ: Ectoplasm composed of fine granules, in alveolar structure. *E. coli:* Ectoplasm structureless. *E. dysenteriæ:* Endoplasm shows large granules in alveolar structure. *E. coli:* Endoplasm composed of much smaller granules. *E. dysenteriæ:* Nucleus stains much less intensely than *E. coli.* *E. dysenteriæ:* Amount of chromtain also much smaller and often no nucleus can be demonstrated.

Reproduction.—Both reproduced by simple fission; also *E. dysenteriæ* reproduces by process of budding. Portion of nucleus is extended, chromatin is diffused in protoplasm, and eventually collects in small clumps around the periphery of the organism. These are then given off with portions of protoplasm as young amœbæ. *E. coli* reproduces after cyst formation. Eight small amœbæ are formed within the cyst which breaks down and liberates them.

"These organisms are so easily distinguished that there should be no difficulty in differentiating them by experienced laboratory men."

Our own observations have had the advantages accruing from the study of cultures of amœbæ. These cultures have included stems from the dysenteric intestine, from the stools of patients without diarrhœa, and from water, vegetables, and other extraneous sources. After careful observation of more than one hundred cultures from these different sources, and extensive experimentation on animals, together with a study of the parasites in stools and tissues, we can not avoid questioning the justification for establishing the two species, *"E. coli"* and *"E. histolytica."* We do not at all question the multiplicity of both genera and species of amœbæ, both within and without the intestines of men, but it does seem as if more work should be done before any classification can be substantiated.

Whatever may be said concerning the general classification, when it comes to the establishment of a *harmless* species, *"E. coli,"* and a *pathogenic* one, *"E. histolytica,"* the supporting data given are insufficient, as will be shown when the pathogenicity of amœbæ is discussed.

METHODS OF REPRODUCTION AND LIFE CYCLE.

It seems fairly certain that there are at least three, and perhaps four methods of reproduction of amœbæ, but whether or not some or all of them are common phenomena of the same amœba, or whether they are different in different species it is difficult to determine.

In cultures, simple fission is easily observed and it is a common property of all amœbæ which we have studied. A second phenomenon, somewhat resembling the above, but which is much more rarely seen, is that of *budding,* by which a small portion of the amœba is separated from the parent parasite apparently without interfering in any way with the motility or other evidences of life of the original organism. In more than one instance we have seen two of these buds given off in succession before we lost sight of the parent.

A third form is that following encystment of the parasite.[5] Encystment, under certain circumstances, is the property of many, if not of all, amœbæ, and of course further reproduction occurs from the cysts. In many cases this certainly takes place by some process of sporulation, sporogony, or schizogony. In others another method, to be mentioned below, appears to result.

We have never been able actually to observe sporulation, although cultures of encysted amœbæ shortly after transplantation to fresh media, contain vegetative forms which often are exceedingly small and numerous.

Apparently another method of reproduction, or revegetation, occurs from the encysted stage. In this a single, motile amœba escapes from the ruptured cyst. We have observed this phenomenon a number of times in cultures, and believe the observation to be accurate. A number of changes take place before this process is completed, and among them we have noted an increased activity of small granules within the encysted amœba, and these granules, while still in active motion, become arranged around the periphery, while the nucleus becomes more prominent. The cyst wall then changes to a less dense and more transparent consistency. Finally, it ruptures and a motile amœba escapes. The old cyst wall remains for some time as a foreign body in the culture medium.

Lastly, we have cultivated two amœbæ which do not encyst even in cultures several months old. One of these has now been in artificial media for about two years and only lately has begun to form imperfect cysts.

MORPHOLOGY, CLASSIFICATION, ETC.

In addition to differences in the methods of reproduction, other distinctions between amœbæ have been described from time to time. These relate to practically everything which may be observed from the study of any one of these parasites. They refer to:

(*a*) *Amœbæ as a whole.*—Size, color, shape, and motility. Number, size, shape, and contents of pseudopodia.

[5] Perhaps there are other forms of reproduction as well.

(*b*) *Ectoplasm.*—Density. Tenacity and dictinction from endoplasm. Color and refraction. Contents, granules, and other elements.

(*c*) *Endoplasm.*—Presence of, absence of, or variation in size and number of granules. Color and refraction. Presence or absence of red blood corpuscles and other formed, foreign elements. Number, size, and contractility of vacuoles. Spore-appearing bodies.

(*d*) *Nucleus.*—Location, shape, size, and variations in the amount of chromatin. Presence or absence of nuclear membrane and nucleolus.

In order to obtain the most complete data, it is necessary as far as possible to study the above characteristics in all stages of the life cycle of the parasites.

(d) *Amœbæ as a whole—Size.*—The dimensions of amœbæ vary between wide limits, but probably from 3 to 40 μ in diameter would be the minimum and maximum of size in cultures, and this variation is almost, or quite equalled when the parasites are observed in stools and in the tissues with amœbic lesions. In the naturally encysted stage, the variation is much less, being from about 8 to 30 or perhaps 35 μ. The explanation of the differences in measurements between amœbæ in the encysted and in the vegetative stages is as follows:

All amœbæ are smaller when they are encysted than when in motion. The smallest sizes, often seen in the vegetative stage—that is, the ones below 8 to 10 μ in diameter—are almost surely young parasites, and these do not naturally encyst until they are older and larger. Under certain adverse conditions, such as an extreme lowering of temperature, even the smallest and youngest forms may be driven to assume a cystic appearance, and, of course, under such circumstances they are exceedingly small. However, this change is not a true cyst formation, but *microscopically* it may be impossible to distinguish from real encystment.

Formerly the variation in size of amœbæ was considered to be of importance in determining species, and especially in separating the so-called pathogenic from the non-pathogenic types. However, more recent writers have attributed less importance to it, contenting themselves with the statement that *"E. histolytica"* is in general larger than *"E. coli."* In our former work we have remarked that measurements of the parasites could certainly not be used in the determination of species. However, after more extended experience, it begins to appear as if measurements, if they could be made with parasites of equal age and in the same environment, might become an important factor in determining species. The trouble, of course, would be the difficulty of being sure of the age and environment of the amœbæ.

With cultures grown from a single amœba, and hence pure, the individual examples vary much in size, particularly in the vegetative stage. In the encysted stage this variation, although present, is much less when amœbæ on the *same plate culture* are considered. Between one culture and another of the same amœba there may be a greater difference,

which, under certain circumstances, may be quite decided. This variation illustrates the difficulty or impossibility of securing a constant environment. Therefore, until the subject is investigated further, the size of amœbæ can not be used as a diagnostic character of species and the value of such determination in differentiating between so-called pathogenic and non-pathogenic varieties is of still less importance, as we will point out in another part of this paper.

Color.—The shades of amœbæ, which vary from greenish to dull grayish, have been noticed almost from the time of their discovery. The variation has been explained in many ways and has repeatedly been pointed out as an aid in the determination of species.

Schaudinn and his followers have again brought this question into prominence in reference to the classification of these protozoa, by regarding the difference in color as one of the important distinguishing points between the harmless and the pathogenic species of amœbæ which they found in the human intestine. The variation in color in amœbæ is very noticeable, but we are not prepared to accept such differences as being of any value in determining species; *rather it is an index of environment.*

In frequent examinations of the stools from a single patient, we have often noticed the increased greenish refractiveness taken on by the parasites as the character of the stool changed from the watery, diarrhœal, blood-free discharge of the early stages to the dysenteric stool of a more established infection. In the bloody, mucous stools the greenish coloration of amœbæ is often quite pronounced, but as the case progresses favorably under treatment, with the disappearance of blood this color is gradually lost and during convalescence we may observe amœbæ which are dull-grayish in color and of an opaque appearance. Again, in addition to the variation in color due to, or intimately associated with the absorption of blood serum or other hæmoglobin-containing substances by the parasites, we have variations which apparently depend upon the presence of bile in the environment. In cases of dysentery, upon the progressive disappearance of bile from the fæces, we have repeatedly noticed the gradual change to a dull-grayish color, by amœbæ which before that time had shown a most marked, greenish refraction. Amœbæ seen in liver and other abcesses usually conform more closely to the color accredited to *"E. coli"* than they do to that assigned to *"E. histolytica."* Other influences may also play a part in this phenomenon observed in amœbæ from the intestine and other parts of the human body. However, the incontrovertible arguments against the supposition that certain colors or differences in refraction are of diagnostic importance in distinguishing between different species of amœbæ are found when we work with cultures of these parasites.

In cultures, amœbæ may be changed from one color to another. These colors are as different as any which have been mentioned in the literature, and this phenomenon can be repeated almost at will by such simple

variations in environment as the addition or subtraction of blood, blood serum, bile, certain types of bacteria, etc.

In view of the above observations it is perfectly evident that amœbæ found in a healthy intestine, or even in one in the early stages of disease, would be very likely to have a grayish color, regardless of the pathogenicity of the rhizopods.

Shape and motility.—All amœbæ assume a great variety of shapes when in motion but when at rest, or encysted, they are invariably spherical or slightly oval. The oval shape is well marked and fairly constant in cultures of our amœbe "V;" this rhizopod also has an unusually prominent nucleus. Motility varies greatly, even in cultures of pure species, and from our observations it seems to depend, at least partially, upon physical laws. It may be quite rapid, or very slow; continuous, or, more frequently, intermittent. An alternating protrusion and withdrawal of pseudopodia may continue to take place for some time, without any change in the position of the amœba. This period of comparative rest *may* be followed by one of great activity, but more usually it precedes the resting or encysted stages.

Although progression is usually associated with the protrusion of pseudopodia, quite active motility can at times be observed without such protrusion. This progression is accomplished by a protrusion of the whole side of the amœba, which is followed by the remainder of the parasite in what appears to be a kind of rolling process; this motion may be kept up by an individual for some time, to be followed later by the usual pseudopodial motility. On the other hand, the parasite may encyst, without again showing true pseudopodial formation. In this class of amœba one is particularly apt to notice currents of the protoplasm which are usually of a vortical nature, formed within the parasite.

The actual distance traveled by amœbæ and the rate of locomotion may very closely be determined from cultures, by inoculating in the center of a plate containing the media, as was described in our first publication, and then watching the rate of spread toward the edges of the Petri dish. These measurements permit us positively to state that a great variation exists in the rate of locomotion. In cultures of some amœbæ, the surface of the media will be traversed in from forty-eight to sixty hours, while in others, during three or more days only a small area will be covered before encystment of all the parasites takes place.

To judge from these observations, if rate of locomotion be of value in determining species, then such determinations are not in harmony with the other characteristics which are given for the differential diagnosis of *"E. coli"* and *"E. histolytica."*

The formation of *pseudopodia* is probably a common property of all amœbæ, and the size, shape, number, etc., of these protrusions may vary between wide limits.

In size they range from almost thread-like projections to large, oval

bodies which constitute one-third or more of the central mass of the amœba. Their shape varies from the perfectly sharp-pointed spinous type of Celli and Fiocca to a very broad, flat, oval, or even to a narrow, undulating type, which may run around the surface of the amœba, having very much the appearance of the undulating membrane of a monad, just prior to its encystment.

The number of pseudopodia which may be present on an amœba at any one time may vary from one to five or more, and frequently pseudopodia pertaining to a pseudopod may be seen.

Whatever the type or the number of pseudopodia may be, their general structure and contents vary but little. The outer coating is formed by the ectosarc, and the contents consist of a portion of the fluids of the parasite alone, to which, as the projection grows larger, may be added portions of the endosarc, and sometimes vacuoles, foreign bodies, etc., and, very rarely, the nucleus of the amœba. In some instances, the endosarc follows the ectosarc in its protrusion so closely that a line of demarcation can not be distinguished and, indeed, it may seem as if the ectosarc was being pushed out by the endosarc.

One very important point, which we have not seen noted elsewhere, is the change in the elasticity of the ectosarc itself during the formation of the pseudopod. The portion of this organ which covers the pseudopod is often much thinner and correspondingly denser than it is in other places, and with very large pseudopodia or where several are present, this stretching of the ectosarc may be sufficient to increase considerably the linear measurements of the surface of the amœba itself.

This fact may be brought out quite distinctly by staining the moving amœba with a very dilute solution of neutral red, which sharpens the distinction between ectoplasm and endoplasm, without in any way interfering with the motility of the parasite. This phoenomenon may also be confirmed by a study of parasites which have been quickly fixed and stained with their pseudopodia well formed.

The character of the pseudopodia varies just as much in cultures of pure species of amœbæ as it does in those which are not pure. One of our first amœbæ obtained in culture, while showing only lobose pseudopodia in the stool from which it came, when it was first grown on artificial media developed perfect, spinous pseudopods, and later would at times develop the spinous and again the lobose type. In the intestinal contents of animals inoculated with it, only lobose pseudopodia were observed, but in subcutaneous abcesses produced by inoculation of similar cultures the spinous variety was frequently present.

(*b*) *Ectoplasm.*—This elastic structure surrounds the outer portion of the amœba. It varies in thickness from about one-third of the diameter of the parasite to a very thin streak, almost a line. Its distinctive appearance is probably only completely developed in the full-grown parasite.

In general appearance it is usually somewhat more dense than the endoplasm, particularly on its outer border; it may appear as a homogenous structure, or again may present a granular appearance without color, or may be of a grayish or greenish tint. Its association with the internal parts of the amœba may be so close that no line of demarcation can be made out, or again it may stand out with perfect clearness, entirely separated from the endoplasm.

In young amœbæ of whatever species the distinction between ectoplasm and endoplasm is rarely demonstrable, and even in adult types or in encysted forms this may be very difficult. In old, encysted amœbæ the density of the ectoplasm appears to increase and its contraction causes a wavy appearance which, together with the slight, brownish color which may also be present, may with age give a picture comparable with that seen in *ascaris* eggs. (Fig. 29, in Publications of the Bureau of Government Laboratories, Biological Laboratory No. 18.) At times, it may seem perfectly structureless, or thicker and denser on the outer margin, shading off toward the center of the parasite, or in some instances it seems to have a sharp, well-defined inner membrane as well as an exterior one. Often, particularly in this latter type, it contains a few or many granules of assorted sizes, distributed in its substance.

This part of the amœba is probably a much more complex structure, both histologically and functionally, than it is generally accredited to be. Numerous observations tend to show the correctness of this conclusion. At times, during the vegetative stage of the amœba, it allows the passage into and out of the parasite, of bacteria, red blood corpuscles,[6] and of other foreign, formed elements of considerable size, and again, during the encysted stage of the organism, it proves to be almost, or quite, impervious to the most powerful staining reagents.

It is a fact that no satisfactory staining reagent for encysted amœbæ has been found. The ectoplasm may be most intensely stained by almost any common dye, but there is very little penetration to the interior structures. On the contrary, this is not true in the vegetative stage, and an almost equally large number of stains may be used, which will even color the interior structures more intensely than they will the ectoplasm. This, in our opinion, depends more upon variations in the ectoplasm than upon changes in the other structures of the parasite with reference to susceptibility to stains.

Further corroboration of this fact is found by a study of the action of dilute solutions of neutral red upon amœbæ. Solutions of this substance, so dilute that the color in a drop can scarcely be detected by the eye, when

[6] We must not forget here that it is possible that these bodies never actually penetrate the ectosarc, but that folds of this membrane envelop them, so that they are only apparently in the amœbæ, in a manner comparable to the relation between the peritoneum and the abdominal organs.

allowed to come in contact with motile amœbæ are quickly taken up in quantities sufficient to stain various internal portions of the amœba a decided pink color, without at all staining the ectosarc. In resting amœbæ this process is much slower and less complete, and here, in addition, the ectosarc is usually stained. However, in encysted types this stain does not penetrate to the interior of the parasite, but may slightly color the cyst wall. When, at times, one is fortunate enough to see a ruptured cyst in the culture, the neutral red, entering through the opening, colors the contents and often the inner portion of the ruptured ectosarc. This observation tends to show that the variations in staining are due to permeability of the ectosarc rather than to changes in the internal structure of the amœba and further indicates that the outer portion of the ectosarc itself is more dense and resistant than the inner.

A further most interesting phenomenon with neutral red solutions may sometimes be noted in motile amœbæ, in which the first granules to show color may be those in a persistent pseudopod. If this is due to the thinned portion of the ectosarc being more permeable, which appears to be the case, it might also further indicate that, in addition to the function of locomotion which the pseudopodia possess, we must consider them as playing a part in the metabolism of the parasite—such as the absorption of food.

Some, at least, of the numerous phenomena of the ectosarc may be explained by physical laws and their application to the age, environment, etc., of the parasite. However, in addition to this we probably have changes due to vital metabolic phenomena and perhaps others due to variety in species.

(*c*) *Endoplasm.*—The endoplasm is probably composed of a network and of fluids in which are embodied the nucleus, vacuoles, spores, granules, and other vital parts of the parasite, often together with red blood corpuscles, bacteria, and other foreign bodies. The proportionate amount of endoplasm as compared with the whole parasite varies with its environment and the stage of its life cycle in which the observation is made. Apparently its quantity is smallest and its density greatest in the encysted stage of the amœba. In the small, young parasite it appears homogeneous, and stains easily and intensely, but at later stages it often begins to show granulation, vacuoles, foreign bodies, etc., until in the encysted condition of the amœba it often appears to be contracted and more sharply distinct than at the beginning. It may or may not be distinguishable from the ectoplasm; sometimes the distinction is well marked and the outer margin may appear almost as if surrounded by a limiting membrane. There may be no apparent color in the endoplasm, or, again, various shades of dull-grayish or greenish refraction may be noticed. This variation in color and refraction has already been discussed. Amœbæ from cultures may be made to take on the so-called "diagnostic," greenish refraction with almost as much certainty as they

will take on a pink tint after the introduction of very dilute solutions of neutral red.

The granular material of the endoplasm may be entirely absent or abundant, and the variation in size of the granules is also decided. These bodies, if present, are not visible in the encysted amœbæ. Their composition and significance is not understood, but in some instances, at least, they appear to be chromatic. They probably have some function in the metabolism of the parasite and they may eventually be of value in species determination. Vacuoles are never seen in the young amœba nor in the encysted one. One or more, however, of different sizes may be present in the adult parasites, and they are usually most numerous just before encystment.

Quite frequently these vacuoles contain numerous, small, actively motile granules. The significance of the vacuoles is not fully understood. However, Mouton, working with cultures of amœbæ, showed that one of the vacuoles was contractile and that this one contained a digestive ferment. Vacuoles are more constantly present and more numerous in cultures of some amœbæ than they are in others.

The inclusion of red blood corpuscles, bacteria, and other foreign-formed elements under certain circumstances by some amœbæ is a rather frequent phenomenon, and one difficult to understand. However, as has been stated in some of our previous communications, it is probably largely a mechanical process. In cultures, amœbæ are often not tolerant of solutions containing blood, but by starting with very small amounts and gradually increasing the percentage of blood in the medium a condition may be obtained in which the parasites will engulf red blood corpuscles apparently exactly as they do in the intestine. This observation is facilitated if blood poor in hæmoglobin be used. The process, whatever its significance, positively is not confined to one particular morphological type of amœba; it can not be used as a differentiating characteristic between pathogenic and non-pathogenic species, and finally the oft-repeated statements that red blood corpuscles are digested by the amœbæ is without foundation in fact. The damage which such cells manifest when extruded from amœbæ, as they always are before encystment, has not been produced by any digestive process but, aside from some probable changes due to the mechanical action of the parasite, are damages which were present when the cell was ingested. We have watched amœbæ containing red blood corpuscles for hours without seeing any evidence of a specific action of the amœbæ upon the corpuscles.

It is simply absurd to employ a small difference in the number of red blood corpuscles which two different amœbæ may happen to have within them, as a factor in species diagnosis. This is emphasized when we remember that it is not very uncommon to find one, two, or even as many as four red blood corpuscles in one of the common monads in a dysenteric

stool. The reasons governing the inclusion of red blood corpuscles by amœbæ will be discussed in the chapter devoted to the pathogenic character of the parasites.

Bodies which appear like spores may often be seen in the endoplasm of adult amœbæ and in the resting and encysted forms. At times, the arrangement and general appearance of the bodies strongly suggest their being spores, which they probably are, but at other times the picture is not so clear. Whatever the nature of these bodies, they apparently have to do with reproduction, but as we have not been able to follow Schaudinn's observations, nor to establish definitely their course and action, we do not desire to discuss the subject further at this time.

(*d*) *Nucleus.*—A nucleus is probably present in all amœbæ, but in some it is difficult to see it and in others impossible to do so, and further, in the case of amœbæ, it may at one time easily be observed and at another not found. It may be spherical, oval, vesicular or irregular in outline; located centrally or excentrically in the parasite; it varies considerably in size and in the amount of contained chromatin. A nuclear membrane may be very distinct or barely visible and there may or may not be a visible nucleolus.

It will be remembered that one of Schaudinn's strongest points of differentiation between *"E. coli"* and *"E. hystolytica"* (except the distinctions based upon the modes of reproduction) was founded on observations upon the nucleus. In *"E. histolytica"* the location was always eccentric, the shape round, of small size or not visible, the chromatin small in amount and the nuclear membrane indistinct. In *"E. coli"* the picture was almost the complete opposite.

These observations are not altogether true when pure cultures of a single species of amœbæ are considered. The variation in the characters mentioned in such pure cultures is sufficiently great to make at least their diagnostic importance questionable. That Schaudinn's conclusions are not always true in regard to amœbæ in sections from lesions in the bowel and liver may be amply observed by any one sufficiently interested to study such sections.

In *summarizing* this mass of fact and theory concerning the biology of amœbæ, it is readily seen how difficult it is to systematize the points in such a way as to justify classification. We have failed to follow Schaudinn or others in their species determinations, and it appears that many important premises upon which their conclusions were based are not borne out by our work. It seems to us that more work must be done before a satisfactory classification of these protozoa can be made, and until such a time we prefer and believe that we are fully justified in retaining the name *Amœba coli* Lösch, to represent those amœbæ which are found in the intestines of human beings.

The bearing these observations have upon the pathogenicity of amœbæ will be more fully discussed in the appropriate part of this paper.

III. CULTIVATION.

METHODS AND MATERIAL.

Our general plan and technique for the cultivation of amœbæ has not materially been altered since our first communication on this subject.[6] A few changes in the details have been made, most of which have been designed to meet special conditions. These are brought out in the next two chapters, in which pure cultures and symbiosis are discussed.

Up to the present time we have cultivated over two hundred stems of amœbæ from water, soil, vegetables, fruits, and other extraneous sources; from the stools of man and other animals; from the dysenteric ulcers examined post-mortem; and from various experimental amœbic abscesses in animals. We have also, in cases of amœbic cystitis, grown the parasites from human urine and twice from liver abscesses in man. In the latter cases there were both bacteria and amœbæ in the abscesses, and, in one case, cultures made from the discharges after operation were only successful on plates which had previously been inoculated with cultures of the bacteria found in the liver abscess.

No difficulty, as was mentioned in the first paper, has been experienced in cultivating any amœba from water, soil, or other extraneous source. However, the problem with those from the human or other animal intestine is not always so simple, and in a certain number of such cases we have even been entirely unable to grow the parasites. *The most important point to be considered in this connection is that this statement is equally true no matter whether the colon infection is a natural one or whether the amœbæ were originally introduced in the intestine from cultures in which they were multiplying profusely.* To a certain extent at least, this difficulty is brought about by inability to produce satisfactory symbiosis; a further discussion of the causes underlying this phenomenon will be given in the appropriate portion of this paper.

We have found that, whereas when first isolated the parasites thrive best in neutral or slightly alkaline media, this preference can be altered by carefully and gradually increasing the acidity, and by this method the amœbæ eventually can be made to propagate in surroundings in which the percentage of acid is greater than it is ever found to be in the normal stomach; it is also true that encysted amœbæ, after days of exposure to quite strong acid solutions, will again develop when they are placed upon satisfactory media.

This renders it evident that vegetables and other substances contaminated with amœbæ can not be rendered harmless by treatment with weak acids and that the ingestion of acids can not be relied on as a prophylactic measure. This statement is rendered the more positive by the fact that our experimental work has shown that such measures are of even less

[6] *Publications of the Bureau of Government Laboratories*, Manila (1904), No. 18.

avail against the protozoa in the encysted state, and this latter phase of their life cycle is in reality the most dangerous one, even if it is not the only period in their existence when they are a serious menace.

PURE CULTURES.

Pure cultures of amœbæ, which will continue to propagate in media free from other living microörganisms, have not been obtained. That this fact is due to difficulty or inability to free the amœbæ from other associated organisms without injury to the protozoa, has been stated by others, and we formerly tentatively concurred in the belief, but this position must now be abandoned.

Perhaps the easiest and simplest method is to get the protozoa in culture with some delicate bacterium and then place the cultures aside until all the bacteria are dead. Bacteria grow very poorly on media suitable for amœbæ, and some of them, such as *Spr. cholerœ*, pneumococci etc., die very quickly under such surroundings unless frequent transplants are made. This process may be hastened by still further reducing the amount of nutritive substance in the amœbæ medium.

Other methods by which we have succeeded in obtaining amœbæ pure and free from bacteria on artificial media are as follows:

Sometimes, in old, encysted cultures, the bacteria which are present may be destroyed by heat and the amœbæ still retain their viability. This is shown by transplants when they are in this condition made to media which contain a satisfactory symbiotic bacterium. The bacteria in an old, encysted culture may sometimes be destroyed, without killing the amœba cysts, by the careful use of certain antiseptic solutions, such as benzoyl-acetyl peroxide, succinic peroxide acid, formalin vapor, chloroform, etc.

In some instances success in separating and obtaining living amœbæ, free from bacteria, can be obtained by using the plate methods described in our first paper for securing amœba together with a pure culture of bacteria. For this purpose the original method is only altered in the particular that the bacterial rings, after being made on the plate and the organism allowed to develop, are then killed by exposing the plates to a temperature below the melting point of the agar. The plates are then inoculated in the usual manner within the ring, with the mixed cultures. Occasionally, under these conditions, the amœbæ not only grow through the dead bacteria but also leave the living ones behind them, and in this manner may be found encysted at a sufficient distance from living bacteria, to allow of their being removed with a platinum loop.

Amœbæ free from bacteria may also often be obtained by the injection of the mixed cultures into the livers of monkeys or subcutaneously, and then inoculating subsequent animals with the contents of the abscesses thus produced until finally an abscess is obtained in which the amœbæ are found to be bacteria free.

The most satisfactory method is the one which consists in allowing the bacteria to die out of a culture, leaving the encysted amœbæ behind.

In considering the above-mentioned means of separating living amœbæ from their symbiotic organisms in cultures, it must always be borne in mind that the amœbæ *encyst* under such conditions and do not multiply.

By these methods numerous working cultures of amœbæ, in large numbers and free from bacteria, may be obtained. That the amœbæ are free

from other organisms is easily shown by plate cultures, and that they are capable of further multiplication is also easily demonstrated by transferring them to media inoculated with a satisfactory symbiotic organism. Multiplication will then be renewed.

Upon a few occasions we have succeeded in obtaining some slight multiplication by transferring the organisms from such pure cultures to other sterile media, but the reproduction in all cases has been very limited and it always entirely ceases after a few transplants have been made, whatever the source of the amœba or whatever the medium employed; nor have we been more successful in using various special media, some of which contained various enzymic and other organic extracts. Details of all these experiments would be tedious and unprofitable, but the following brief summary indicates the scope and extent of this line of work:

(1) Amœbæ were grown from a head of cabbage, and in order to demonstrate whether or no an *extract* of this cabbage would be a medium for amœbæ free from bacteria, the cabbage was rapidly macerated with sterile water and filtered through a porcelain filter. The result was negative. Again, such an extract, inoculated with the bacteria in symbiosis with the amœbæ, kept for twenty-four hours and then filtered, would not nourish the amœbæ.

(2) Amœbæ from liver abscesses and the intestine would not thrive in various filtered extracts from human and animal livers, although these proved excellent culture media for bacteria.

(3) Various extracts from, and emulsions of, bacteria failed to prove satisfactory media even where the symbiosis between the amœbæ and the living bacteria of the same stem was all that could be wished. Some encouragement in this direction was obtained in the following way:

Amœbæ growing in symbiosis with *Spr. choleræ* seemed at first to be splendidly nourished by the cholera vaccine prepared by autolytic digestion according to R. P. Strong's method.[7] When the living bacteria were also present even in small numbers, this substance certainly exerted a most favorable influence upon the amœbæ, but with bacteria free cultures such effects were very transitory.

(4) Liver and other animal tissue extracts obtained in a sterile manner furnished no better results than the filtered products.

(5) Various preparations of blood and the serous effusions of man and animals proved unsuccessful.

(6) Gelatin cultures of *Spr. choleræ* after liquefaction, killed by heat or chloroform, were not satisfactory media for the amœbæ which were propagating with the living *Spr. choleræ* of the same stem.

IV. SYMBIOSIS AND SYMBIOTIC ORGANISMS.

EXTRANEOUS OR SAPROPHYTIC SYMBIOSIS.

Amœbæ in water, soil, on vegetables, or in any other location outside the animal body, have never been found except in association with other microörganisms.

The variety of such organisms is very great, including bacteria, yeasts,

[7] *Publications of the Bureau of Government Laboratories, Biological Laboratory,* Manila (1903), No. 16.

and other parasites, and this flora in many instances is constantly subject to changes caused by the deposition of other varieties and by dust and rain. Even in large bodies of water, like the Manila city supply, the bacterial flora changes from time to time, but amœbæ are almost always present.

In all these situations there undoubtedly exists a symbiosis between the amœbæ and some (but not necessarily all) of the other micro-organisms present. This association is not as fixed as it becomes in amœbæ after cultivation; because the changing flora makes it necessary for this symbiosis also frequently to change. Work with cultures has shown positively that amœbæ are capable of such alterations provided they are brought about gradually.

The proof that a symbiosis with other organisms is present in all conditions where amœbæ are found outside of certain situations in the bodies of the higher animals and that this association is often a changing one which necessitates constant readaptation of the amœbæ to new conditions, is important because of the bearing it will have in the study of the higher symbioses. Most of this proof will be furnished when symbiosis in cultures is discussed, but a few points may properly be emphasized here.

If pure cultures are made of all the different organisms found in a body of water which, for example, contains the usual assortment of amœbæ and bacteria, amœbæ from the original source will grow well in some of these cultures, poorly in others, and not at all in the remainder.

If this experiment be repeated several times with the same cultures and the same water, it will sometimes be found that divers bacteria will form the best cultures at different times or that amœbæ will develop on two or more widely different bacteria at the same time. This fact most probably indicates the presence of different strains of amœbæ—that is, amœbæ varying in the sense that the symbiosis is different and that they may be found in the same body of water at the same time. It is further emphasized by the following experiment:

Plate cultures of all the cultivable bacteria, etc., are made from a body of water, which also contains amœbæ. Four large flasks of the water are now taken and sterilized to kill all microörganisms, including amœbæ. Two of these flasks are inoculated with the bacteria originally grown from the water and the other two left sterile. After standing a few hours to allow the bacteria to develop, the flasks are inoculated as follows:

One sterile flask and also one of those containing bacteria, with a small quantity of water from the original source (which water contains both amœbæ and bacteria). The other two flasks are inoculated with water from some second source, which water also contains amœbæ.

The amœbæ from the first water will develop in both flasks in which they have been inoculated, whereas those from the second source will often develop only in the sterile flasks. At times, the natural symbiosis of the amœbæ from both sources is so similar that they will develop in

all the flasks, and again, at times, nothing will develop in the flasks inoculated with bacteria because the symbiotic organism has been lost[8] in working with the mixed cultures.

CULTURAL SYMBIOSIS.

In this connection we have little that is new to add to the statements made in our first publication. In cultures, as is the case elsewhere, excepting in stages in which the amœba is absolutely parasitic, the symbiosis of these protozoa with other living microörganisms is constant and definite.

In mixed cultures of bacteria with amœbæ, the symbiosis may easily be proved to exist with but one strain of the organisms which are present. In some cases, where the amœbæ have recently been isolated from extraneous sources, the selectiveness of this symbiosis may not be so marked, and again, even from these sources, the association may be so sharp and definite that the addition of certain organisms to the culture will destroy the amœbæ, notwithstanding the fact that an abundance of their symbiotic germs remain in the culture.

This antagonism of amœbæ in culture to certain bacteria was noted in our first publication, and has been repeatedly verified since. This was first brought out sharply by our discovering that one of our amœbæ refused to grow in the presence of *B. violaceus manilæ* Woolley, no matter what its symbiosis was at the time the bacillus was introduced into the culture. However, later, by close attention to the methods previously described for altering the symbiosis in cultures, this amœba and *B. violaceus* were grown together in pure symbiosis. We have had similar experiences with several amœbæ and with different bacteria since the above was published, and in each instance the original, apparently marked antipathy between the amœbæ and bacteria could be overcome. Repeated and carefully controlled experiments have fully demonstrated the accuracy of these observations, and in addition have developed some interesting points in the character of this altered symbiosis. Both the specific character and the property by which amœbæ may change their symbioses in culture are very important points which, as we shall presently prove, have a very suggestive bearing upon the process of amœbic infections in animal tissues.

While a satisfactory association with some living microörganism seems essential to the propagation of amœbæ in artificial media, it does not appear that such organism is used as a food by the parasites, but rather that some product of metabolism due to the association, or some enzyme produced, is what actually nourishes the amœbæ.

In young, healthy cultures of amœbæ and any bacterium, the germs, upon examination of either fresh or stained specimens, are rarely found

[8] That is, it has become overgrown or destroyed in some other way.

within the amœbic protoplasm. This observation is further borne out by the fact that in any culture the most active amœbæ are found along the margins of the culture where the bacteria are also not so numerous.

However, when the cultures are older, and in the more advanced portions of plate cultures which are two or three days old, where both amœbæ and bacteria are more numerous and where many of the amœbæ are older and the bacterial reproduction less active, we often do see amœbæ containing bacterial bodies. Again, amœbæ in culture may be made to take up red blood cells, granules, and foreign matter of various types. This phenomenon is most frequently to be seen in the type of old amœbæ which has just been mentioned and, as with the bacteria, it seems to us that this process of engulfing visible matter is rather due to a lack of selectiveness shown by degenerating amœbæ than to a type of assimilation connected with the nourishment of these protozoa.

Amœbæ, filled with bacteria and other foreign matter, may often be seen rapidly to disintegrate under the microscope, and before encysting they invariably extrude all of these foreign bodies.

INTESTINAL SYMBIOSIS.

(*a*) *Saprophytic.*—There can be no reasonable doubt but that specific and definite symbiosis plays as great a part in the propagation and development of amœbæ in the colon as it does in the test tube, and it is true that the intestinal symbiosis is a changing one due to frequent alteration in the bacterial flora of the bowel, just as it is in the altering bacterial growth in water or vegetable substances. The microörganisms forming the intestinal flora during health are largely saprophytic, and in this respect the flora does not differ largely from that of other sources in which amœbæ are found.

Therefore, it would seem that the propagation of amœbæ which reach the colon alive would depend largely upon whether satisfactory symbiosis could be found there or, in other words, whether there might be present in the bowel, at the time of the entrance of the amœbæ, a sufficient number of bacteria similar to those forming the protozoan's last extraneous or test-tube symbiosis to continue its nourishment until it could adapt itself to the changed environment. Or again, there might be carried along with the amœba upon its entrance into the gastro-intestinal tube enough of its former intimate surroundings to allow rapid multiplication to take place and to establish foci of its last symbiosis in the bowel, before destruction of the parasites could take place.

It would seem that when a satisfactory symbiosis has been established in the bowel by either of the above methods and when amœbæ are propagating in the intestinal tract, their multiplication might continue indefinitely, and under certain conditions which we do not understand this condition might exist without any injury to the intestine. Just to what extent this actually occurs it is extremely difficult to determine.

Up to this point we have been discussing what might be called saprophytic or artificial symbiosis in the bowel, and in this connection it makes no difference whether the amœbæ which are introduced are obtained from the head of a cabbage or the amœbic liver abscess of a dysentery patient, whether they are "*E. coli*" or "*E. histolytica*," and whether they are to multiply for a while with their symbiotic bacteria and finally pass out of the bowel or whether they are later to develop a true amœbic ulceration.

(*b*) *Parasitic.*—We will now discuss the second type of intestinal symbiosis, which is the connecting link between all which has been brought out on this subject and true parasitism or animal tissue symbiosis, which is next to be considered.

In this type, through changing symbiosis and other metamorphoses, we reach the condition in which amœbæ, instead of multiplying in the lumen of a healthy bowel are, by the aid of or association with bacteria, growing and propagating in lesions of the intestinal wall, or perhaps, while having the same bacterial symbiosis, in liver or other abscesses.

The exact method by which these changes are brought about is not understood any more than are the causes of changes in the virulence of bacteria, *but that such changes from amœbæ and bacteria in a normal intestine to the same amœbæ and bacteria in amœbic lesions in the bowel and elsewhere occur, and finally that this evolution may proceed to the elimination of the bacteria, while amœbic lesions are still produced in animal tissues, is established beyond the shadow of a doubt.*

We are fully cognizant of the importance of the statement that amœbæ may change from a saprophytic to a parasitic existence, but after several years of careful experimentation we do not see any other interpretation of the results. Whether the amœbæ in question were ever true saprophytes may be doubted, but many of them surely came from sources in which there was no apparent evidence of present or of past parasitic environment, and we have never yet worked with an amœba which could not be changed from a bacterial association to one with an animal tissue. There is much in recent literature which bears upon this subject.

For example, we can quote R. Koch's explanation of the specificity of certain trypanosomata for specific animals, and Theobald Smith's thoughtful communication upon the biology of the tubercle bacillus. Smith believes that the parasitism of the tubercle bacillus is best and most often developed in the lungs, because, after entrance the tubercle bacillus at first has a more or less saprophytic environment in which to propagate, and in this way it is able to survive for a sufficient length of time gradually to take on a more parasitic environment, due to close association with the animal tissues.

Whatever our interpretations of our findings may be, it is a fact that amœbæ which probably have been multiplying indefinitely in a bacterial symbiosis may: (1) continue indefinitely in such bacterial environment, (2) they may change from a bacterial to a mixed bacterial and tissue

symbiosis in the intestine or liver, and (3) this change may be altered to true parasitism by the elimination of the bacteria, and tissue invasion and destruction by the parasites still take place.

TRUE PARASITISM.

We do not frequently find amœbæ, without the presence of other living microörganisms, propagating in and associating with definite lesions in animal tissues and it is probable that such conditions, when they do occur, are examples of errant types or other unusual circumstances.

In all intestinal lesions, and in many liver and lung abscesses, there are present, in addition to amœbæ, one or more varieties of bacteria, and cultural experiments demostrate that under these circumstances a definite bacterial symbiosis often exists between the amœba and some of the organisms which are present, whereas at the same time the amœba exists in partial tissue symbiosis. In other instances, even in mixed amœbic and bacterial lesions, the association of the bacteria seems no longer to be necessary for the life and action of the amœbæ, for no symbiosis with any of the bacteria which are present can be found by cultural methods. It is probable that, in these cases, the amœbæ have taken on a symbiosis with the animal tissues; in other words, have become true parasites and that the presence of the bacteria is without significance.

In naturally acquired amoebic infection in animals, the parasitism rarely goes beyond the compound bacterial and amœbic stages referred to above. However, true parasitic infections free from bacteria, do occur in natural infections and they may be produced artificially.

Evidences of true parasitism in natural infections are found in bacteria-sterile abscesses of the liver, brain and other portions of the body, and in two instances, upon which a special report will be made at a later time, we have found true amœbic septicæmia. In one of these cases the organisms were associated with bacteria and *P. westermanii* in multiple abscesses, but in the other no other microörganisms could be cultivated from the circulating blood which contained the amœbæ.

In this latter case, amœbæ were found in the blood from the median cephalic vein and from the juices of a swollen leg, the swelling of which we believe to have been due to amœbic thrombosis. Amœbæ were also encountered in the small abscesses which developed on the skin of the leg. The organisms from the circulating blood remained alive in cultures for some time, but would not grow on transplants.

ALTERATIONS IN SYMBIOSIS.

It has already been shown, both in our first paper and in this one, that satisfactory symbiosis is necessary for the propagation of amœbæ whether they are in a mud puddle or in an abscess of the liver. It is also established that this symbiosis is often a changing one in natural surroundings and that variations in environment may be made at will in

cultures by following simple requirements. Furthermore, it is probable that the conditions governing successful changes in symbiosis in artificial media are very similar to those taking place in nature and that conclusions drawn from such changes are sound. If living animals are used instead of culture media, alterations in symbiosis within their economy are just as apparent as they are in the test tube. It is by changing symbiosis that true parasitism, with its far-reaching results, is established. However, at present the important point is to show that amœbæ of various morphologic conditions and from many sources may be deviated from saprophytic surroundings to true parasitic environment; it is also desirable to determine whether or not this process is reversible.

(*a*) *Increasing processes.*—An amœba from water, soil, vegetables, animal intestines, or some other source may be cultivated in pure species with bacteria, as has been described in our previous paper. This amœba may then be obtained with a pure culture of bacteria and this bacterial symbiosis may be changed at will from time to time. Under certain circumstances, previously described cultures of this class may be fed to monkeys or to man, and amœbic infection of the colon result. Such cultures may also be injected subcutaneously or into the livers of monkeys, and compound amœbic and bacterial abscesses result. The amœbæ may again be cultivated from both of the above sources. If they are taken from the intestine, the original bacterial symbiosis may be satisfactory, but if the infection is of several days' standing then some other bacterium from the monkey's intestine may be necessary. This shows that while the symbiosis is still bacterial, it has changed from the one originally introduced. The amœbæ may also be recultivated from the mixed liver abscesses, if the bacteria are still present, but in this case the bacteria which were introduced with the amœbæ will be found the most satisfactory symbiotic organism, and usually the only one with which growth may be obtained.

If the liver abscess is allowed to remain undisturbed for some time or still better, if the contents of such an abscess is used to produce a second one by direct inoculation into another monkey, the cultivation of the amœbæ becomes more difficult and after a time is rendered impossible, notwithstanding the fact that the original symbiotic bacterium may be still present in the pus and that this organism is the one used on the culture media.

We therefore conclude from these results that, as in other instances, the symbiosis has changed in the liver abscess, and as there are no microorganisms present other than the ones which were introduced, we also conclude, and experimentation proves this to be correct, that the change in symbiosis in this instance is one which has been formed with the liver tissues—that is, represents true parasitism.

In other words, notwithstanding the fact that the original bacterium which was introduced is still present in the abscess, it is now no longer

necessary for the life of the amœba. The latter has become a true parasite and is living at the direct expense of its host. If an amœba is grown with a bacterium such as *B. typhosus*, which is capable of producing a bacteriæmia in monkeys, and if such a culture is injected into a monkey two or three days after the animal has previously been infected with the same strain of *B. typhosus*, then we often obtain multiple, localized abscesses, which are sometimes so extensive as to suggest a general infection. Often such abscesses will contain amœbæ and *B. typhosus*, but at times only the rhizopodia will be found. Amœbæ from such abscesses may usually be recultivated in symbiosis with *B. typhosus*. This, together with the necessity of a previous bacterial inoculation of the animal, shows that notwithstanding the fact that the lesions which are produced are anatomically amœbic, bacteria are still playing a part, though a very indefinite one, in the symbiosis.

If the contents of such abscesses as these are injected directly into a second, healthy monkey, there may or may not be a single abscess at the place of injection but, contrary to the results of the first experiment, no extensive infection is observed; but if, as with the first, this second monkey has previously been given a typhoid bacteriæmia, then the same far-reaching infection may result. However, there is one difference in the second monkey, namely, the presence of bacteria is much less evident, and it may be impossible to reclaim the amœbæ by culture. In a third or fourth monkey, inoculated directly from the abscesses in the second or third, the previous bacterial inoculation may be dispensed with, and rather extensive infections, free from bacteria, may sometimes still be obtained. In other words, a true parasitism on the part of the amœbæ has been established.

Such infections may be transmitted from animal to animal during two or three transfers, rarely through more. The amœbæ can not be cultivated from these sources by using our present means any more than they can be from the bacterially *sterile* liver abscesses in man.

The facts outlined in the above general statement of the building up of the pathogenicity of amœbæ have been repeatedly verified by many and large series of experiments which seem to us to be conclusive. One series is given here and others will be found summarized in Chapter V:

No. 1.—Monkey (*M. cynomolgus*) inoculated in the peritoneal cavity with a suspension of an encysted culture of amœbæ and the bacteria cultivated from cabbage.

No. 2.—Monkey (*M. cynomolgus*) inoculated directly into the liver with part of the culture used in No. 1.

After seven days both animals were killed. No. 1 was normal and No. 2 showed an abscess of the liver, from which amœbæ and bacteria were reclaimed by culture.

No. 3.—Monkey (*M. cynomolgus*) inoculated in the abdominal cavity with an encysted culture from the liver abscess of monkey No. 2. After seven days the animal was killed and found normal at autopsy.

No. 4.—Monkey (*M. cynomolgus*) inoculated in the abdominal cavity with an encysted culture of the original cabbage amœbæ in pure symbiosis with *B. typhosus*.

No. 5.—Monkey (*M. cynomolgus*) inoculated directly into the liver with the same culture as that used for No. 4.

At autopsy, one week later, No. 4 was found to be normal and No. 5 showed a liver abscess from which amœbæ and *B. typhosus* were obtained by culture.

No. 6.—Monkey (*M. cynomolgus*) was inoculated in the abdominal cavity with an encysted culture from No. 5.

No. 7.—Monkey (*M. cynomolgus*) was given, subcutaneously, 2 cubic centimeters of a bouillon culture of *B. typhosus* and twenty-four hours later was inoculated in the abdominal cavity with the same culture as was used to inoculate No. 6.

At autopsy, one week later, No. 6 was found to be normal with the exception of a small abscess in the abdominal wall, and No. 7 showed multiple abscesses in the omentum and liver, and one in the lung. These abscesses contained amœbæ and bacteria, but only the *B. typhosus* grew on culture.

No. 8.—Monkey (*M. cynomolgus*) inoculated in the abdominal cavity with the contents of an abscess occurring in the omentum of No. 7.

No. 9.—Monkey (*M. cynomolgus*) which had eighteen hours previously received 2 cubic centimeters of a bouillon culture of *B. typhosus* was inoculated in the same manner as No. 8.

At autopsy, four days later, both these monkeys showed amœbic abscesses in the omentum, but the infection was much more extensive in No. 9.

(*b*) *Decreasing the virulence; avirulence.*—Another consideration in this connection which is second in importance only to that brought out by the power of amœbæ to increase in virulence under proper environment, is the question of the loss of virulence by these protozoa.

In discussing this portion of the pathological biology of these organisms, both the question of symbiosis and of direct virulence must be kept in mind, just as it is when the increase of virulence is considered. We have already shown the great difficulty, and often the impossibility, of causing amœbæ to return to a condition at all like their former mixed environment, when once they have been grown on solid media in pure cultures of bacteria. This is even true when all the known facts concerning symbiosis are borne in mind and are used in attempts gradually to induce the parasites to return to their former mixed association. Pure, mixed cultures may occasionally be transferred from solid to liquid media in the same pure symbiosis, but attempts to introduce other organisms and thus to have mixed bacterial cultures usually result in the disappearance of the amœbæ.

Similar results are obtained when working with symbioses in the higher animal tissues. Although amœbæ may be changed from symbiosis with one bacterium to that with another in cultures, after animal infection has been brought about, growth on artificial media is usually only possible with the organism which was present in the amœbic lesion in the animal. If the animal infection is continued for a sufficient length of time to allow symbiosis with the tissues or juices of the body to be established, then

the amœbæ are not reclaimable by culture, even though the exact bacterium which may still be present in the lesions is used for symbiosis, and when the strict amœbic lesion *free from the bacteria* is established it is *usually* impossible to grow the amœbæ from it by any of the known methods.

Similar results are obtained in a study of virulence. Just so long as it is possible to cultivate an amœba with its associated bacteria, no matter how active it may be in animal tissues, its virulence may be decreased upon artificial media.

One of our most virulent amœbæ (11524 of our first report), when last used at the time that report was written, would, in combination with *Spr. choleræ,* produce very active amœbic lesions. After nearly two years of cultivation on artificial media, it was apparently non-virulent and, in order to restore it to its original condition, its virulence had to be reproduced as in the first instance. Before the restoration of its virulence it would not produce lesions in animal tissues; afterwards it would.

This susceptibility of amœbæ to a change of environment is of great practical importance in its application to naturally contracted amœbic infection. If our conclusions are correct, the most dangerous amœbæ are the ones which live saprophytically in mixed environment, because the gastro-intestinal tube is surely rich in bacterial and other organisms and amœbæ of the kind mentioned above would be the only ones which would be likely to form or to find satisfactory symbioses in such a place. *Such symbioses are necessary and must precede a more parasitic existence in the intestinal lesions.*

It is extremely difficult, to judge from our observations, to understand how an amœba which has once become a strict parasite, or even a very selective one in mixed, tissue lesions, can be able to thrive in the mixed environment into which it is necessarily thrown upon leaving the body. Such amœbæ would be extremely dangerous if their transmission were a direct one or if the conditions were nearly to approach such a case; but when they are discharged into water, sewage, etc., their tenure of life is probably ended, and, while these statements are not proved, it is at least possible that in considering prophylaxis in amœbic infection the more virulent types derived from lesions in animal tissues are the least to be dreaded.

V. PATHOGENESIS.

It may be remembered that in our first publication the literature relating to the important question of the pathogenesis of amœbæ was summarized under four headings, as follows:

"(1) Amœbæ are harmless; (2) they intensify or alter lesions already present (they are secondary invaders); (3) there are pathogenic and non-pathogenic amœbæ; (4) all amœbæ are, or may become, pathogenic.

At that time we concluded that none of the first three views had been established beyond criticism and that pending a complete solution of the matter, the fourth was the only safe one to consider from the standpoint of public health in the Tropics.

Before entering upon a discussion of our continued work on this subject, a few communications which were not considered in the first paper or which have appeared since, will be reviewed. The most important article which deals in part with the pathogenesis of amœbæ is that of the late Prof. F. Schaudinn, which appeared before our first paper, but which was not accessible to us at that time. The other recent papers have largely been confirmatory of Schaudinn's observations.

The conclusions of Schaudinn which bear upon the subject under discussion were based upon the following observations: (1) Amœbæ, always corresponding to the description of *E. coli*, were found in 20 to 66+ per cent of the stools of healthy people (people without clinical manifestations of diarrhœa) which he examined. (2) These amœbæ, when injected in the recta of kittens, did not produce disease. (3) Amœbæ corresponding to his description of *E. histolytica* were only found in the stools of people with clinical dysentery. (4) These amœbæ when injected into the recta of kittens produced dysentery. (5) "By studying fresh sections of the intestine infected with *E. histolytica* it is easy to observe the amœbæ pushing themselves between the epithelial cells of the mucosa."

Charles F. Craig, in San Francisco, found *E. coli* in 65 per cent of the stools of healthy people and in 50 per cent of those of patients suffering from diseases other than dysentery, and he has informed me verbally that he has found them in the stools of 70 per cent of healthy soldiers examined in Manila. His published conclusions confirm and elaborate Schaudinn's. They are:

(1) The intestine of man may be infected with two varieties of amœbæ, a pathogenic (*E. dysenteriæ*) and a non-pathogenic one (*E. coli*). (2) *E. coli* is found in 65 per cent of healthy intestines and in 50 per cent of those having other diseases, if a saline cathartic is given. (3) These organisms can easily be distinguished in both fresh and stained specimens. (4) They differ widely in their manner of reproduction. (5) *E. dysenteriæ*, when fed in milk or injected into the recta of kittens, produce typical amœbic ulceration. (6) The same procedure (5) with *E. coli* gives negative results. (7) Similar experiments with the intestinal bacteria alone do not produce dysentery

It will be noticed that, with the exception of Schaudinn's statement under subhead No. 5, there is nothing in any of these observations or conclusions which has not already been stated many times by different writers. They were also summarized in our first publication. The statement of Schaudinn in paragraph 5 is a most remarkable one and open to criticism.

We have already dealt extensively with all the points brought out in these new investigations,[9] but as Schaudinn's work has attracted so much attention it seems justifiable again to discuss some of his important conclusions.

Granting for a moment that amœbæ may be present in the healthy colon, how can this fact warrant the conclusion that these amœbæ are

[9] *Loc. cit.*

harmless or of a non-pathogenic species? Malarial parasites may be found, upon repeated examinations, for months in the blood of individuals living in malarial zones, without chills and fever, or without any other recognizable departure from health; trypanosomata may be present for long periods of time in apparently healthy cattle, carabaos, guinea pigs, or rabbits; filaria may be observed for years in the circulating blood before clinical symptoms develop; but in these cases no one would argue, for example, that the malarial parasite was a harmless commensal.

In discussing the question whether amœbæ multiply in the normal intestine we wish, before detailing our new work, once more to call attention to our original communication in which we concluded that, while amœbæ may occasionally be found in stools from a healthy person, the fact of their proliferation in a normal bowel for a period greater than the longest incubation period of the disease, had not been proved. In view of the statements of Schaudinn, Craig, and others, that amœbæ are so prevalent in the intestines of healthy people, we have again studied several series of cases occurring in all classes of people found in these Islands, and have been unable to find evidence requiring us to change our previous conclusions. Indeed, we could not in a single examination of the fæces, find amœbæ in 70 per cent of real cases of dysentery, to say nothing of examinations of healthy people.

These series, which have been undertaken to determine the prevalence of amœbæ in the intestine, have not dealt with the question of whether or not this situation is a normal one. The series are as follows:

(1) Five hundred and eighty-seven cases from Bilibid Prison Hospital, including all nationalities, were examined. Amœbiasis is quite prevalent in this prison. Of these 154 contained amœbæ, or 26+ per cent. Of 38 consecutive autopsies from the same hospital during the time the above examinations were being made, 16 or 42+ per cent showed amœbic infection of the colon.

(2) In another series of 100 cases, three examinations were made from one week to ten days apart; of these 39 contained amœbæ, or 39 per cent.

(3) Of 318 American patients in the Civil Hospital, mostly admitted because of some gastro-intestinal disturbance, 57 were positive, or 14+ per cent; among 69 native patients in same hospital, 11 were positive or 17+ per cent.

(4) Of 143 stools of American patients examined in the clinical laboratory of St. Paul's Hospital, by Mr. O. Lindquist, resident pathologist to the hospital, 44 per cent were positive; among 217 native patients, 31 per cent were positive. These examinations were largely among patients suffering with diarrhœa or dysentery.

(5) Of thirty examinations in the biological laboratory by other members of the staff from stools sent in, as a general rule, by private patients suffering from dysentery or diarrhœa, 7 were positive, or 23+ per cent.

In addition to differences in the percentage of infections discovered by stool examination, which differences must at least in part be due to the personal equation when the examinations are conducted among

similar classes of people in the *same country*,[10] we must always consider whether a bowel from which the stool to be examined is taken is diseased or not. As was stated in our first paper, we can only accept data in this connection which have been secured after a careful clinical study supported by autopsy records in each case, because it is only after these precautions are taken that certainty as to the absolutely healthy condition of the bowel can be reached.

Amœbæ may be found in the stools of patients who give no clinical evidence of disease, under the following conditions:

(1) In every case of amœbic infection there is an incubation period, or a time intervening between the entrance of the amœbæ and the production of lesions, during which the organisms may be found in the stools. This incubation period may be several months, as was demonstrated in our original communication, and it is not improbable that it may sometimes be for years.

(2) In many cases of genuine amœbic infection, after careful treatment, the patient apparently and in some instances surely recovers. There is a disappearance of all clinical symptoms, and an autopsy following death from intercurrent disease will establish the fact that a cure has been effected, and yet amœbæ have been found in the stools for months, or even for a longer time. We have repeatedly observed this to be a fact, and R. P. Strong and others in Manila have also noticed it.

(3) Our records show a number of cases in which it is conclusively proved that amœbic infection may occur and yet the patient recover without ever having manifested any external symptoms of the disease during life, the fact of amœbic infection only being confirmed by autopsies performed when the patients have died of other causes. In these cases the organisms would have been found in supposedly healthy persons during life. Indeed, the presence of scars and of other evidences of a healed, antecedent amœbic ulceration of the colon is quite a common post-mortem finding in general autopsy work in the Philippine Islands.

(4) Another class of cases resembling the one just described is that in which the infection is slight and the recovery so complete that even a subsequent autopsy would reveal nothing abnormal. That this may be the case is proved be our experience with the other class which has just been described, in which the diagnosis is only possible after an autopsy made possible by death due to an intercurrent disease which occurred during the mild, amœbic infection.

(5) There is the considerable percentage of cases of true amœbiasis in which, because of the location of the lesions, the extremely chronic character of the disease and for other reasons, long periods of time have elapsed during which but slight or no clinical manifestations of infection were evident. The absence of diarrhœa or dysentery in these instances is noticeable. This type of infection is so frequent that we shall shortly report fifty cases, with autopsies.

(6) Finally, it is probably true that in a certain number of cases amœbæ continue to multiply in the human intestine for days, months, or possibly years without ever producing ulceration in the bowel. Such cases, if they do exist, are not of great frequency and it would be difficult, or impossible, to separate them during life from the five groups mentioned above.

Even if such cases were more frequent and if it were easy to establish their existence it would seem much more rational to attribute the failure to infect to the

[10] The probable influence of climatic conditions, particularly upon the white man in the Tropics, must not here be lost sight of.

lack of a satisfactory symbiosis or environment for the parasite or to the very complex question of tolerance or immunity on the part of the host, exemplified in so many ways in other parasitic infections of man, rather than to attribute this phenomenon entirely to a lack of pathogenicity on the part of the amœbæ.

In discussing the question of the existence of pathogenic and non-pathogenic amœbæ Schaudinn, as we believe largely owing to false premises, has once more gone over the ground covered by the older workers, which was reviewed in our first publication, and he has again maintained it to be possible to divide amœbæ into two types, pathogenic and non-pathogenic.

In the first part of this paper we have noted the zoölogical considerations upon which he established these two classes, and while we are unable entirely to follow his observations, we dislike to take issue with that part of his work, and this is particularly the case because, as we stated in our previous paper, it is not unlikely that several species of amœbæ will probably be found to exist in the intestine, and it is also probable that more than one species may be found to be concerned in the production of lesions in that organ. However, we disagree with the classification of "*E. histolytica*" and "*E. coli*" as being respectively pathogenic and non-pathogenic for the following reasons:

Amœbæ which may be observed in the stools from patients who have no symptoms of diarrhœa or dysentery (the so-called healthy individuals) are not confined in their characteristics to those given for "*E. coli.*" Protozoa answering more nearly to Schaudinn's "*E. histolytica*" are surely to be isolated from the above class of cases, and probably with almost as great frequency as "*E. coli.*" An amœba which we have in culture (11524 of our first paper) at one time was very virulent, producing dysentery when it was ingested by monkeys or man. After about three years of cultivation on artificial media, no lesions could for a time be produced with this organism, although the exact technique of our first inoculations was repeated. This amœba has again become virulent by the manipulations which we have used to produce animal infection with all classes of these protozoa.

It is also true that amœbæ answering partially to the description of "*E. coli*" may, and often are, the only ones to be found in the fæces, in ulcers, or in sections from some of the most severe cases of the infection in man. Cultures of amœbæ of this class can be so manipulated as to produce amœbic ulceration of the colon in monkeys and in man, and furthermore they can be made to produce multiple amœbic abscesses in the lungs, liver and omentum after intraperitoneal inoculation.

One of our amœbæ is a very satisfactory type of "*E. coli.*" It has a prominent central nucleus, it is a slowly moving organism, its ectoplasm and endoplasm are difficult to differentiate, and in many other characteristics it conforms to the type. With this organism, which is one

of our most recent cultures isolated from vegetables purchased in a Manila market, we have produced liver abscesses in monkeys after intraperitoneal inoculation.

ORIGINAL WORK.

In our first publication conclusive evidence was given of the pathogenic character of amœbæ in producing ulcerative colitis in man and monkeys. We now wish to offer more evidence upon this point and in addition to show that the amœba is capable of a very definite parasitism. Furthermore, we wish to demonstrate that morphologically the organisms which produce this colitis may resemble either "*E. coli*" or "*E. histolytica*" as they are described by Schaudinn and that they may be isolated from a variety of sources. It is almost impossible to conceive of man ever before having been in any way associated with some of these sources.

We have used cultures of encysted amœbæ in all of our experiments, because we have long been convinced that these are the most certain to be infectious.

INTESTINAL INFECTION.

SUMMARY OF EXPERIMENTS.[11]

(1) If amœbæ from *any* source are cultivated on artificial media, and the encysted cultures are then fed to monkeys by means of a stomach tube, after a variable time the stools of some of the animals will contain the organisms. The usual symptoms of infection are observed, and at autopsy the lesions and parasites characteristic of amœbiasis will be found. However, in the cases of many of these animals no bad results follow the introduction of the organisms and amœbæ are never found in the stools. In others, after the infection is established, the animal may finally entirely recover and the amœbæ disappear.

In still another class, amœbæ after a certain incubation period, may be found for a time, finally to disappear from the stools without any visible symptoms of infection. However, if the animals are killed, in some of them amœbic lesions will be found in the colon. One monkey which was infected in the manner detailed above developed on amœbic abscess of the liver which was found at autopsy, and in another an abscess of the lung was observed.

The percentage of infections to be included in this class of experiments does not appear to be materially influenced by the type of the symbiotic bacterium which was present in the cultures, nor are the results very different when the amœbæ are in symbiosis with more than one variety of bacteria. If large doses of the symbiotic bacteria alone are first fed to the animals for several days and if then the culture of the amœbæ and the bacteria is ingested, the percentage of infections is increased.

[11] See also *Publications of the Bureau of Government Laboratories*, Manila (1905), No. 18, 70-76.

(2) The percentage of infections is greater than it is in animals fed in the manner given under 1, if *suspensions* of cultures of amœbæ are inoculated directly into the cæca of monkeys by hypodermic injections, either after opening the abdominal cavity or by direct puncture through the abdominal wall.

However, even by this method amœbic ulceration does not always follow, the results being influenced by the same agencies which cause a variation in the feeding experiments.

(3) Amœbic infection can be produced in monkeys by rectal injections of cultures of the organisms, but the results of this procedure are less certain than they are when either of the other two methods is employed. The study of the influences which govern these results has not brought satisfactory conclusions.

We have practically abandoned experiments which depend upon introducing the organisms into the gastro-intestinal canal, because a more accurate method, which we will presently describe, has been developed by us. In the intestine the conditions for the study of amœbic or other infections are very complex and show little beyond the proof that amœbic ulcers may be produced by the introduction of cultures of the protozoa; therefore, experimentation on other organs of the body serves more easily to solve the question of the pathogenicity or non-pathogenicity of the organisms.

Our work in specific regard to the intestine seems to have established the following:

(*a*) Amœbæ are the causative agents in certain infections of the bowel.

(*b*) Bacteria play an important part in this process by furnishing symbioses for the amœbæ during the establishment of a more genuine parasitism.

(*c*) In many of these infections true parasitism probably is not reached in the bowel, for here, even in the amœbic ulcers, the bacteria still help in the formation of a symbiosis. In this respect bacteria may be considered to influence the fundamental etiological agent. Certain of the pathogenic bacteria which are in symbiosis with the amœbæ must certainly also take a more active part in the etiology of the lesions. Combinations with this class of organisms usually influence the symptomatology of the disease and they may always be recognized in the histological study of tissues taken from such cases.

(*d*) It is also true that pathogenic bacteria which are not concerned in the amœbic symbiosis may enter the intestine and play a part in the subsequent course of the amœbic lesions, and therefore it is probable, although it is not proved, that the simplest and purest amœbic lesion of the bowel is one where pathogenic organisms are not present and in which the bacterial symbiosis takes place with a non-pathogenic bacterium.

(*e*) In this connection it must be remembered that the conjunction of otherwise harmless bacteria, in close association with amœbæ, may possibly increase the virulence of the bacteria themselves.

(*f*) Morphologically, the types of amœbæ which will produce lesions in the intestine are not confined to those described as *"E. histolytica;"* indeed, they may as closely follow *"E. Coli,"* and we must therefore conclude that, in so far as pathogenicity and non-pathogenicity are concerned, the species *"E. coli"* and *"E. histolytica"* have not been established.

LIVER INFECTIONS.

The intestinal infection of man and animals surely represents the lowest type (often a mixed one) of the parasitism of amœbæ, just as the lung infection represents the lowest order of parasitism of the tubercle bacillus, as has recently been pointed out by Theobald Smith in his classical article on the biology of the tubercle bacillus. As with the latter, the rhizopod here finds a nearer approach to its former saprophytic surroundings. For these reasons, consumption is the most frequent type of tuberculosis, just as intestinal infection is the most frequent one of amœbiasis. In the case of the latter, this type of infection is the precursor of almost, if not all of those in which a higher type of parasitism is manifested, and which is expressed in the form of liver and lung abscess; abscesses in the brain and in other organs and in the occasional, errant, general infection of which we will shortly publish two cases.

SUMMARY OF EXPERIMENTS ON INFECTIONS OF THE LIVER.

(1) If monkeys are inoculated directly into the liver with mixed cultures of bacteria and amœbæ, abscesses are produced in a certain percentage of the cases. These abscesses may contain amœbæ and bacteria in kind like the ones injected, or again only the amœbæ may be present. Such abscesses are influenced somewhat by the type of bacteria in symbiosis with the amœbæ and in some instances only bacteria are found in the abscesses.

When bacteria, which of themselves will not produce liver abscess, are used in symbiosis, then it is found that decided differences in the percentage of abscesses produced with different amœbæ occur, but the percentage of successful results with any given culture of amœbæ may be increased by certain variations in the conditions. This is made evident by the following summary of our results.

If amœbæ are transplanted directly to culture media from an abscess produced in the manner just mentioned and then injected into the livers of other monkeys, abscesses in the latter are produced with greater certainty. An increase in virulence would therefore seem to be produced by this variation. Similar results are obtained by injecting the contents of the abscess from the first monkey directly into the liver of a second

animal, but if this last direct inoculation from animal to animal is continued, the bacteria are gradually eliminated, and finally bacteria-free amœbic liver abscesses result. However, by using this procedure the percentage of infection is again somewhat decreased, until in every series of animals in which we have tried it, infection will finally no longer occur. This failure to infect is difficult to explain, but as it also occurs in other experiments which produce strict parasitism, we are inclined to look on it as an expression of the errant rôle which absolute parasitism forces upon these protozoa.

On the other hand, if the symbiotic organism is one like *B. typhosus,* capable of producing bacteriæmia in monkeys, and if, from twenty-four to forty-eight hours before the liver inoculations, the animal is injected subcutaneously or intraperitoneally with a pure culture of *B. typhosus,* then a maximum percentage of abscesses is produced and the infection can be kept active from animal to animal, no matter whether the infecting material be taken from subcultures or be transferred by direct inoculation of the pus from one abscess into the liver of the next monkey. In these cases secondary abscesses are also sometimes found in the lungs and occasionally in the spleens of the animals inoculated.

It is more difficult to produce an intestinal infection with subcultures from abscesses produced by any of the above methods than it is with amœbæ which are grown from saprophytic sources or from the intestine. The reasons for this—at first sight rather astonishing—observation are obvious from what has gone before, for although the parasitic character of the amœba has been raised, its selectiveness has also increased to a point where it rarely finds a satisfactory symbiosis in the saprophytic flora of the intestine. It is now easy to understand why the older workers had so much more difficulty in infecting animal intestines with the contents of amœbic liver abscesses than they had with the amœbæ from the dysenteric intestine.

INTRAPERITONEAL INFECTION.

Infection by the injection of cultures of amœbæ into the peritoneal cavity is more difficult than it is by inoculation into the liver, and it is rarely accomplished without some preliminary treatment of the cultures and of the animals. However, with proper care, a most extensive amœbic infection of the omentum and other abdominal structures may be obtained in monkeys and in guinea pigs.

(1) If cultures of amœbæ from various extraneous sources or even from the bowel are injected directly into the peritoneal cavities of healthy monkeys, infection almost never occurs, and this is true regardless of the symbiotic bacteria present, but—

(*a*) If, for example, a culture of amœbæ and *B. typhosus,* which has failed to infect by inoculation in the abdominal cavity, is first inoculated into the liver, and subcultures are then made from the abscess, these sub-

cultures will sometimes produce infection when they are injected into the abdominal cavity.

(*b*) If the subcultures are injected into the abdominal cavity of a monkey or guinea pig which six to sixty hours before that time has been inoculated with *B. typhosus,* then abscesses are much more likely to be formed in the omentum and elsewhere. In one series of experiments they were produced in four out of five monkeys. These infections are sometimes very extensive, many abscesses may occur in the omentum and also in the liver, spleen, abdominal wall and lungs. It is impossible long to continue these infections from animal to animal by employing subcultures, because after the first, or at most the second animal has been used, the amœbæ present in the abscess will no longer grow on artificial media, even in symbiosis with the original *B. typhosus.* However, the infection may be continued through five successive animals by direct inoculation of the abscess contents of one animal into the peritoneal cavity of the next one, the latter having previously been infected with *B. typhosus.* After the second or third animal has been injected, then the preliminary treatment of the monkey by inoculation with *B. typhosus* may be omitted, but the infection can then be continued through no more than two additional animals. We can not explain the failure to continue this high parasitic type of infection by intraperitoneal injections any more than we can similar infections in the liver, but it is probably due to similar causes, which may be the inability of an organism which normally is saprophytic to continue an existence of absolute parasitism.

INTRAVENOUS INFECTION.

Infection occurred in one out of three monkeys which were inoculated intravenously with encysted mixed cultures of amœbæ and *B. typhosus,* obtained from an experimental liver abscess. These monkeys had previously been inoculated with *B. typhosus* alone. In the one positive result, the lesion consisted of two small amœbic abscesses in the lung and one, about 5 millimeters in diameter, in the spleen. We have already encountered two cases of general infection in man. However, in one of these the distribution was the same as that of an associated parasite, *P. westermanii,* and seemed to be due to a lymphatic rather than to a blood distribution.

IMMUNITY.

The influence upon the production of lesions by bacterial immunity possessed by animals against the symbiotic bacteria in amœbic cultures has not, as yet, received the attention the subject deserves.

It may be recalled that it was brought out in our first publication that, whereas abscesses were produced in the livers of a certain percentage of monkeys by the direct inoculation of cultures of amœbæ and *Spr. cholerœ,* that such an abscess did not occur in one monkey which had previously been immunized against that organism. It was furthermore noticed

that no infection could be produced by abdominal inoculation into animals on occasions when the bacteria were destroyed by Pfeiffer's phenomenon. In the more recent work which we have just been discussing, in cases in which the infection of either the liver or abdomen was aided by previously infecting the animal with the symbiotic bacterium (*B. typhosus*), it was found that the best results were obtained if the amœbic inoculation was performed at a no greater interval than twenty-four to sixty hours after the preliminary bacterial infection. Positive results were less certain if several days were allowed to elapse between the inoculations, than when healthy animals were used.

These observations lead so directly to a discussion of the interesting problem of immunity in general against parasitic infections, which we are not prepared to undertake at the present time, that we here only wish to record these preliminary observations on the *influence of a destroyed bacterial symbiosis upon the parasitism of the associated amœbæ.*

THE PRODUCTION OF LESIONS.

Having established the fact of the true parasitism of amœbæ, not only in the production of the intestinal lesions, but also in the liver and other tissues of the body, it becomes important to examine carefully the manner in which these lesions are produced.

Practically all which has been written upon this subject deals with intestinal infection, and the majority of writers consider that the amœbæ penetrate the mucosa in a mechanical manner and reach the deeper structures, where lesions begin. Schaudinn also expresses this opinion and describes how, in fresh sections of the intestine, he has seen *"E. histolytica"* penetrating between the epithelial cells of the mucosa by means of the pseudopodia thrown out by the dense, tenacious ectosarc. Our observations lead us to differ not only with Schaudinn's interpretation of the conditions but also with his conclusions.

In our studies of sections from many cases of amœbiasis we have never as yet seen any evidence of intestinal infection in which the mucosa was intact. The primary lesion is always of the mucous membrane and the epithelial cells always suffer. Of course, because of the extensive undermining which often occurs in the submucosa, one can find areas in which the mucosa is intact over deeper lesions for considerable areas, but lesions of this kind are not early ones, and when examination reveals the point of entrance, in that portion a diseased patch of mucosa will always be found. Indeed, we have been so fortunate as to observe lesions so close to their initial stage that only the mucosa showed changes.[12]

[12] These have been described by Woolley and Musgrave in *Publications of the Biological and Serum Laboratories, Bureau of Government Laboratories,* Manila (1905), No. 32, and also in *J. Am. Med. Ass.* (1905), 45, 1371.

To judge from the facts which have been brought out in this work, we conclude that the manner of invasion is similar to that belonging to other infections. The primary action seems to be due to a toxic secretion which is a product of the amœbic symbiosis, acting upon the exposed cells of the mucous membrane. It is likely that, in addition to considering the chemistry of symbiotic products, we can not ignore the receptive condition of the cells themselves, influenced, as they may have been, by their evironment. It is probable that the mechanism of the intestinal infection does not materially differ from that taking place in the liver and other organs, excepting that it is more complex because of the mixed and changing environment to which the amœbæ are exposed.

In discussing the production of liver abscess it has already been pointed out by Musgrave [13] that something more than the mere presence of pathogenic amœbæ in the liver is necessary before abscess formation takes place. It was shown that the presence of amœbæ in the liver, after the colon has already become ulcerated, must be of very frequent occurrence.

When the data brought out in this paper are considered in this connection, two facts stand out prominently: (*a*) Only a comparatively small portion of actual amœbic infection of the liver results in abscesses or the production of any other changes which we can at this time call specific; (*b*) abscess formation and the bio-chemistry of amœbic lesions in general is a complex phenomenon not yet fully elucidated. Probably it depends, at least partially, upon related alterations which occur both in the parasites and in the involved tissues.

VI. SUMMARY.

In comparing our observations with some of the recent literature upon the biology of amœbæ it appears that the establishment of two species of amœbæ for the human intestine is hardly sustained by the data given.

While the multiplicity of species of amœbæ inhabiting the human intestine is not questioned, it is found by working with cultures of pure species, grown from a single amœba, that variations in some of the characteristics are as great as are many of those given by some authors for species determination.

We have not actually observed the entire life cycle of any amœba, but known forms of reproduction are: (1) Simple fission and less frequently budding during the vegetative stage, and (2) one or more forms of reproduction from an encysted stage either by some type of sporulation, sporogony, or schizogony or by a phenomenon which has been observed in cultures, namely, the escape of a single amœba from a ruptured cyst.

The size of individuals of pure species of amœbæ in culture varies with the age and environment of the parasite. In the same plate culture all ages and sizes are found in the vegetative stage. In naturally encysted

[13] *Phil. Journ. Science* (1906), 1, 552, 553.

cultures the size is more nearly uniform in the same plate, but more variation is found between two or more different cultures of the same amœba.

The color and refractiveness of amœbæ are largely an index of environment and may be altered in cultures by simple methods. In the stools of dysenteric patients such alterations are observed in the changing conditions of the bowel content. Shape, motility, and pseudopodia formation are, at least in part, influenced by physical laws and the environment of the parasite.

The quantity, refractiveness, and relative distinctiveness of the ectoplasm and endoplasm are of doubtful value in the determination of species, and the inclusion of numbers of red blood corpuscles and other formed elements by the endoplasm surely is not confined to one species of amœbæ as classified.

The shape, location, amount of contained chromatin, of the nucleus and the prominence of the nuclear membrane, have been given as important points in differentiating between *"E. coli"* and *"E. histolytica,"* but we have not been able fully to confirm the published observations nor always to harmonize them with other points in species determination.

In summarizing this mass of fact and theory concerning the biology of amœbæ, it is readily seen how difficult it is to systematize the points in such a way as to justify classification. We have failed to follow Schaudinn or others in their species determinations, and it appears that many important premises upon which their conclusions were based are not borne out by our work. It seems to us that more work must be done before a satisfactory classification of these protozoa can be made, and until such a time we prefer and believe that we are fully justified in retaining the name *Amœba coli* Lösch, to represent those amœbæ which are found in the intestines of human beings.

The general plan for the cultivation of amœbæ has not been much changed from that given in our first publication.

Amœbæ may easily be grown from water, soil, vegetables, and other extraneous sources. It is somewhat more difficult to cultivate them from the intestine of man or animals, and this is true whether the infection has occurred naturally or the parasites have been introduced artificially.

From liver and other tissue abscesses, whether occurring in the course of the intestinal infection or produced by inoculation, growth is still more difficult and often impossible.

Pure cultures of amœbæ which will continue to propagate in media free from other organisms have not been obtained. This is not due to failure to free the cultures from bacteria without injury to the parasites, for this may be accomplished with comparative ease.

Amœbæ, outside of certain locations in the human or animal body, are always associated with other microörganisms, and experiments demonstrate that a more or less definite symbiosis exists between the parasites and some one or more of these organisms (usually bacteria).

In the intestine such a bacterial symbiosis is still more definite and select. This specificity of the bacterial symbiosis increases with the parasitism of the amœba until, sometimes in the bowel and usually in liver and other abscesses, the bacterial symbiosis is eliminated and the amœba assumes the rôle of a strict parasite living directly at the expense of the host.

We have been again unable to find amœbæ as prevalent in the stools of human beings in Manila as have been reported for this locality and for other countries during the last few years.

The presence of amœbæ in a healthy bowel is not a convincing argument against the pathogenicity of these parasites, but so far as the Philippine Islands are concerned, the continued propagation of amœbæ of any kind in a healthy bowel is surely an infrequent occurrence. The difficulty of recognizing a "healthy intestine" has been pointed out, and we have discussed some of the conditions which may lead to error in diagnosis.

We must question the justification for establishing a pathogenic and a non-pathogenic species of amœbæ from the data given for the differentiation between *"E. coli"* and *"E. histolytica."* The reasons for this have been fully set forth in the article, some of the important points being as follows: Amœbæ found in the stools of so-called healthy people do not always conform to the descriptions given for *"E. coli."* Amœbæ found in scrapings or sections from dysenteric lesions of the bowel, liver, and elsewhere do not always conform to the requirements for *"E. histolytica."* Cultures of amœbæ answering more nearly the description given for *"E. coli"* can, by methods described in this paper, be made to produce amœbic ulcerative colitis in man and monkeys and abscesses of the liver, omentum, spleen, and lungs in monkeys. In cultures, single species of amœbæ are often found which are characterized by possessing a combination of some of the features which have been described as distinctive for different species. Amœbæ from many extraneous sources, and presumably saprophytic, may be cultivated on artificial media, and with such cultures ulcerative amœbic colitis may be produced in man and animals and abscesses brought about in the liver, lung, omentum, spleen, and muscular tissues of animals.

ILLUSTRATIONS.

In figs. Nos. 1 to 9, inclusive, the magnification in all is exactly 800 diameters. The reader is requested while examining these reproductions to bear in mind the characteristics which have been designated as distinguishing between "*E. coli*" and "*E. histolytica.*"

Figs. Nos. 1 to 3, inclusive, photographs of amœba "γ." This amœba was grown from vegetables and is a pathogenic parasite. With cultures of it we have produced amœbic colitis and amœbic abscesses in the liver, lungs, omentum and spleen of animals.

No. 1. Forty-eight-hour transplant, inoculated from an old, encysted culture.

No. 2. Twenty-four-hour transplant from No. 1.

No. 3. Twenty-four-hour transplant from No. 2.

The amplification in these three pictures is exactly the same (800) and shows the variation in size in a pure species of amœba. This is quite decided between the encysted forms in No. 1 and the vegetative form in No. 3. In No. 1 are shown fully developed cysts, others in which reproductive changes are appearing, and several small vegetative forms which have escaped from cysts. Encysted amœbæ may also be seen, showing the rather dense, prominent ectosarc sharply distinguishable from the endoplasm as in Schaudinn's "*E. histolytica*" but, on the other hand, in the same amœbæ the prominent central nucleus with a distinct nuclear membrane, as in "*E. coli,*" may also be seen. Early reproductive changes are shown in several of the amœbæ, and in these the line of demarcation between ecto- and endo-plasm is less marked. No. 2 shows a cyst containing a single young amœba and another ruptured cyst from which an amœba is about to escape. This phenomenon was also shown in figs. 29 to 32 in our first report. No. 3 represents a vegetative form, probably a young but full grown parasite; except in the large pseudopod which is thrown out, the distinction between ecto- and endo-plasm is not sharp, and the nucleus accredited to "*E. coli*" is also present.

Figs. Nos. 4 to 6, inclusive, photographs of amœba "cabbage." This amœba was grown from cabbage and is a very pathogenic parasite. With cultures of it any of the lesions described in this paper may be produced.

No. 4. Forty-eight-hour-old transplant, showing young vegetative forms and an early cyst.

No. 5. Twenty-four-hour transplant from an old, encysted culture, showing four old cysts and two bunches of young amœbæ freshly liberated.

No. 6. Vegetative form of figs. 4 and 5. The ectoplasm is prominent in the encysted forms in figs. 4 and 5, and in the fresh cyst in fig. 4 the differentation from endoplasm is distinct. None of these amœbæ show a nucleus. In the vegetative form of the same amœba in fig. 6 we have the prominent nucleus described for "*E. coli*" as well as the lack of differentiation between ecto- and endo-plasm.

Figs. Nos. 7 to 9, inclusive, photographs of amœba "11524." A description of this amœba will be found in our first report. It was grown from the stool of a dysenteric patient.

No. 7. Old, encysted culture, showing distinction between endo- and ecto-plasm and absence of nucleus.

No. 8. Transplant from No. 7, showing two cystic and three vegetative forms.

No. 9. Transplant from No. 8, showing full grown vegetative form. The spinous pseudopodia formation, which was distinct in the specimen viewed under the microscope, is barely visible in the photograph. The nucleus which was very distinct is slightly out of focus.

In this connection attention is invited to figs. 7 to 11, inclusive, of our first report, which are also reproductions of this same amœba.

Fig. No. 10. Twenty-four-hour-old transplant from growing culture of an amœba from a dysenteric stool. Amplification about 1,200. Showing very prominent nucleus with heavy nuclear membrane and nucleolus situated *eccentrically.* The distinction between ecto- and endo-plasm is usually very sharp in this amœba, and its motility is as a rule very sluggish.

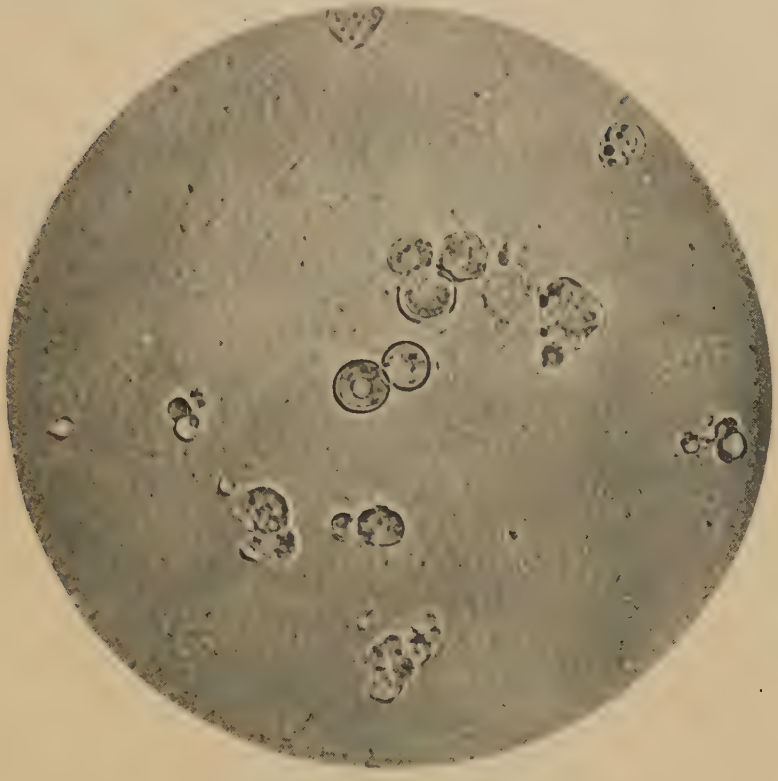

FIG. 1.

FIG. 2.

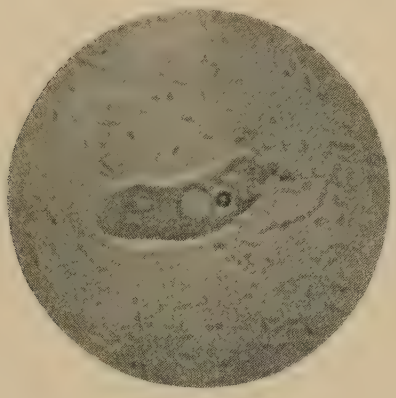

FIG. 3.

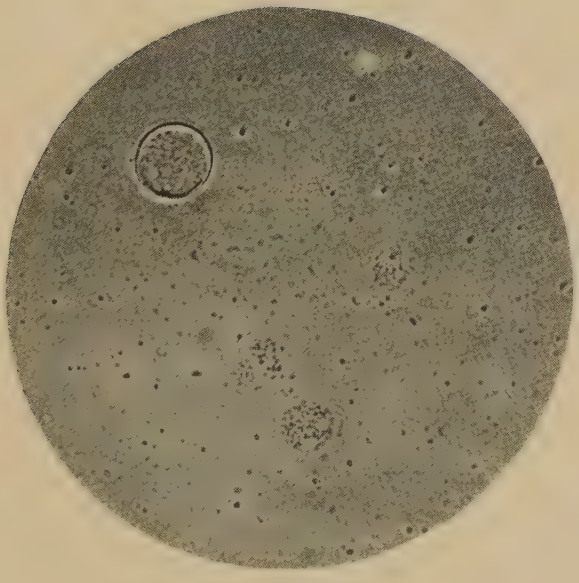

FIG. 4.

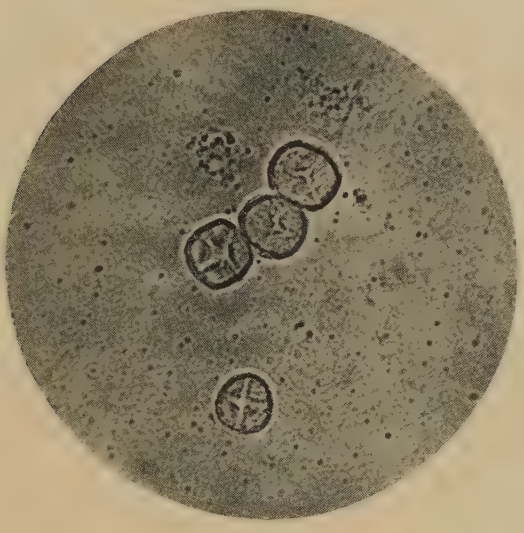

FIG. 5.

FIG. 6.

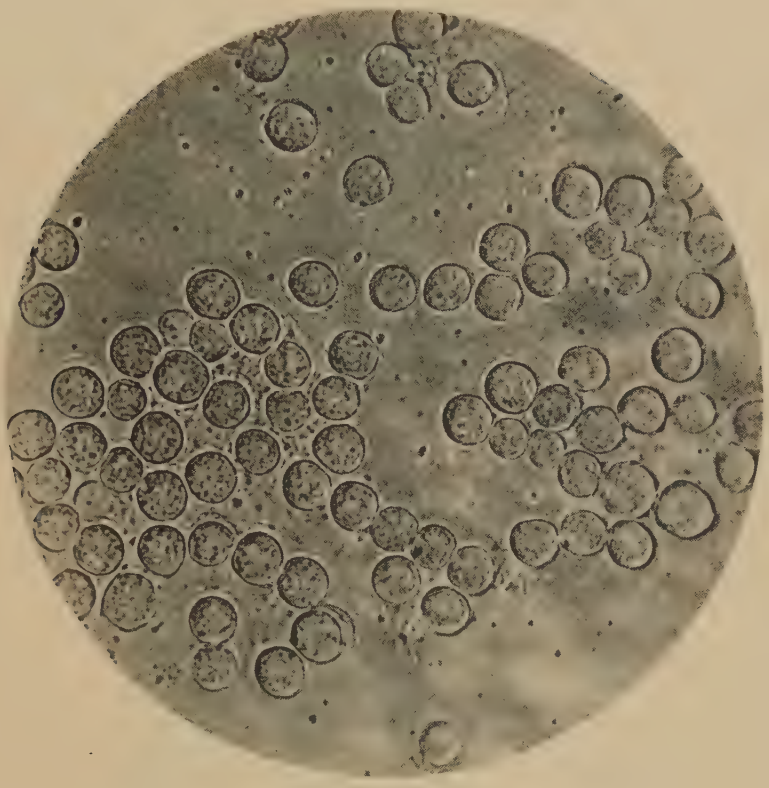

FIG. 7.

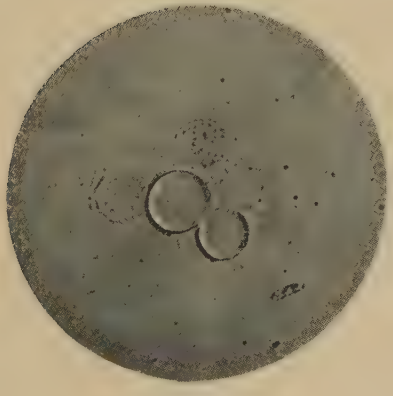

FIG. 8.

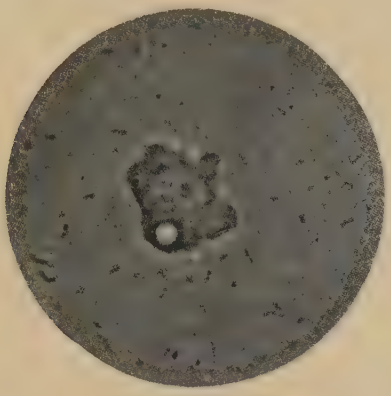

FIG. 9.

FIG. 10.

THE TYPES OF BACILLI OF THE DYSENTERY GROUP.[1]

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INTRODUCTION.

The etiological importance of *Bacillus dysenteriæ* (Shiga) as the causative factor in dysentery was first proved in Manila by Flexner (1), Strong (2, 3), and Musgrave (3), and Strong strictly distinguished between two types of dysentery, viz, the bacillary and the amœbic. The latter type, as a rule, pursues a chronic course and amœbæ can generally be discovered at all times in the stools, whereas the former is an acute disease, the causative factor of which is *Bacillus dysenteriæ*. Bacilli of apparently identical characteristics were later isolated from dysenteric stools in Europe, Asia, and America, so that to-day the etiologic importance of *Bacillus dysenteriæ* in sporadic, endemic, and epidemic dysentery has become fully recognized.

Subsequently, in the year 1900, when the papers of Flexner, Strong, and Musgrave had already been published, Kruse (4) reported on an epidemic of dysentery occurring in Laar, Germany.

At that time Kruse thought he had encountered an organism which differed from the Shiga bacillus. This led to a further discussion of the subject of differentiation by Shiga and by Flexner, who claimed motility for *Bacillus dysenteriæ*, and by Kruse who had never observed this phenomenon in any of his cultures. In June, 1901, Kruse (5) contributed a second paper in which he concluded that Flexner's bacillus was probably closely related to, but still a variety of the species he (Kruse) had isolated, and, although he had not examined Shiga's cultures, he believed from the similarity in the description between Shiga's and Flexner's organisms and from the geographical situation of Japan and the Philippines that the two cultures were identical, and consequently that his own and Shiga's bacillus were very similar organisms. In this same paper he also reported on epidemic dysentery in insane asylums and from some cases of this nature he isolated organisms which could not be distinguished morphologically or culturally from the genuine dysentery bacillus, but which differed from it in their serum reactions. By a careful comparison of their action with the

[1] Read before the Section of Bacteriology, Hygiene, and Infectious Diseases of the Japanese Medical Congress, March 4, 1906.

serum of dysenteric patients and with immune serum from animals, he not only showed that these organisms differed from the dysentery bacillus he first isolated but that they apparently constituted a second variety or species.

Therefore, Kruse was the first who drew attention to the variations in the agglutinability of the dysentery bacillus. On the basis of this distinction, and in spite of the similarity in morphology and in all cultural properties, known at that time, between *Bacillus dysenteriæ* and the organism isolated from cases of institutional dysentery, he proposed to designate the latter as *pseudo dysentery* bacillus.

The investigations of Spronck (6) seemed to confirm this distinction, he having isolated a similar organism from patients suffering with dysenteric symptoms in Utrecht (Holland), in which, however, the clinical manifestations were not so typical as those encountered in acute, epidemic dysentery. However, Flexner (7) and later Vedder and Duval (8) opposed this view and emphasized the fact that the variation in agglutinability was only one of degree and that there was agreement in other properties.

In September, 1902, Duval and Basset (9) reported on the etiology of the summer diarrhœas of infants. From such cases they isolated an organism which they believed to be identical with *B. dysenteriæ* not only in morphology, cultural features and pathogenesis, but also in its reaction with specific serum. Park and Dunham (10) next isolated an organism which produced indol in peptone solution and which, while it agreed in its agglutinative reactions with the Flexner strain found in Manila, differed in this respect from the one Shiga obtained in Japan. Hiss (11) and Klopstock (12) endeavored to differentiate dysentery bacilli from typhoid and colon bacilli by taking advantage of the distinct reaction in fermentation.

The studies of Lentz (14) and of Hiss and Russell (15) further emphasized the cultural distinctions between the different dysentery bacilli and the variations in their agglutinative reactions. These investigators found that two types of bacilli could be distinguished, one which fermented mannite and the other which did not cause acid production in this medium. The agglutinative reactions of these two types indicated further that the so-called "pseudo-dysentery" bacillus regularly fermented mannite, while the other, the original type of *B. dysenteriæ*, was unable to act upon that substance.

Martini and Lentz (13) a short time after, made additional studies upon the agglutination of these two varieties of dysentery bacilli, in which they employed the serum of a goat immunized to the "Shiga" strain of the bacillus. Their results are interesting in showing that such a serum agglutinates only the "Shiga"-"Kruse" epidemic variety in high dilutions, but that some other strains of dysentery bacilli (all of which do not attack mannite) agglutinated in lower ones of 1:25 to 1:50. On the basis of this difference in agglutination and in behavior toward mannite, they uphold the distinctions proposed by Kruse between the so-called "dysentery" and "pseudo-dysentery" bacilli.

Park and Carey (16) called attention to the fact that all varieties of dysentery bacilli (obtained from dysentery cases), which produce indol in large amount and which develop acid from mannite, distinctly differ in their agglutinative reactions from those which do not act upon mannite and which produce no indol, or only a trace of that substance.

Gay (17), in a study of the types of dysentery bacilli in relation to bacteriolysis and serum therapy, repeated the experiments of Lentz and of Hiss and Russell

and confirmed their results as to the action of the bacilli on mannite. As regards the bacteriolytic action of serum upon the two types, he showed that the bacteriolysis in vitro takes place when correspondence exists between the type of bacilli and of immune serum, irrespective of their strain, and it fails to occur when the types are not in conformity. This view was also supported by Martha Wollstein (18), who, in the examination of the stools of one hundred and fourteen infants suffering from infantile diarrhœa found dysentery bacilli in thirty-nine cases. Thirty-eight of these fermented mannite and maltose and produced indol, while one, obtained from the most severe case, did not ferment mannite, thus agreeing with the "Shiga" type.

Park, Collins, and Goodwin (19) in May, 1904, published a paper dealing with the group of dysentery bacilli and the varieties or organisms which should be included in it. The results of their agglutination and absorption tests indicate that the bacilli isolated by themselves and others may be separated into at least three types or varieties. These display differences in their fermentative characters, and the grouping thus arrived at substantiates the classification based on the fermentation test which was made by Hiss. The first type is represented by the original "Shiga" strain. None of the bacilli belonging to this class produce indol (with the exception of a trace) and they do not ferment mannite, maltose, or saccharose. The organisms composing the second type ferment mannite with the production of acid, but do not split maltose or saccharose in peptone solution or produce indol. The bacilli of the third type approach nearest to those of the colon group, since they not only produce indol and actively ferment mannite but also act energetically upon pure maltose and feebly upon saccharose. Animals injected with each of these types develop specific immune bodies and agglutinins, all the bacilli agreeing in cultural characteristics. In consideration of all the facts, they add that it seems incorrect to designate the mannite fermenting groups as pseudo-dysentery bacilli.

Hiss (20) by comparing the fermentative action of the different strains on the five carbohydrates (dextrose, maltose, saccharose, dextrin, and lactose) and alcohol mannite, succeeded in giving what seemed a reliable classification and this division also accorded with the observed agglutinative phenomena. His conclusions are as follows:

(1) The bacilli of dysentery fall into four major groups which have fermentative and agglutinative characters distinguishing them from one another. However, sub-groups, due to differences in agglutinative characters alone, may occur among cultures having the same fermentative functions, so far as has been determined.

(2) The four major groups are as follows: The *first*, represented by the Shiga-Kruse bacillus, ferments dextrose readily and at times maltose, after many days; the *second* one, represented by Hiss's "Y" bacillus, ferments dextrose and alcohol-mannite (maltose and saccharose may be fermented but not with ease); the *third*, represented by Strong's Philippine culture, ferments dextrose and mannite with ease, saccharose is fermented with comparative readiness (and maltose at times slowly); the *fourth*, represented by Flexner's Manila culture, ferments dextrose, mannite, maltose, saccharose, and dextrin with ease.

(3) Agglutination and absorption tests show that the agglutinative characters of these different groups are specific.

The American observers are generally inclined to consider the so-called acid and non-acid types of dysentery bacilli to be of equal etiological importance and some of them regard the differences between the two

types as insignificant, but in spite of the observations of all of these authors, the German observers have usually considered the Kruse type as the only one which has a causal relationship to acute dysentery. I think this view is held because of the fact that in Germany no attempt has been made to observe the fermentation reactions more in detail or more completely to compare the agglutinative and bacteriolytic characters of the different strains.

Lentz (21) in his last paper on this subject, although recognizing the acid bacilli as probably being the cause of a variety of acute, epidemic dysentery, agrees with Jurgens (22), Morgenroth (23), and other authors in refusing to consider these bacilli in the same group with the so-called non-acid bacilli (Shiga-Kruse strain). He says, in his contribution to Kolle and Wassermann's "Handbuch der pathogenen Microörganismen":

Nach den Untersuchungen von Gay, Park und Carey, besonders aber nach den neueren Forschungen von Morgenroth, Jurgens sowie Deycke und Reschad Effendi, gewinnt es immer mehr an Wahrscheinlichkeit, dass auch der von Flexner auf den Philippinen gefundene Erreger der Ruhr angesprochene Bacillus als selbständiger Krankheitserreger neben dem Shiga-Kruseschen Ruhrbacillus auf der Erde weit verbreitet und im Stande ist, Ruhrepidemien hervorzurufen.

But again he adds:

Auf diesen Umstand (d. h. Agglutinationsverhältnisse) ist auch die irrige Annahme von Shiga, Kruse, Flexner und Foulerton zurückzuführen, dass die von Shiga, Kruse, Flexner und Strong gefundenen Stäbchen untereinander identisch seien, ein Irrthum, zu dem sie dadurch verführt wurden, dass sie Rekonvaleszentenserum zum Nachweiss der Identität dieser kulturell sehr "ähnlichen Bacterien" verwandten.

However, after considering the results of my own experiments, which will be related in this paper, I can not regard Lentz's view as correct, nor can I see any reason for considering any organism which causes dysentery to be a pseudo-dysentery bacillus or for the justification of the use of the terms acid and non-acid dysentery bacilli.

Instead of accepting the views of Lentz and of Kruse, it seems rather that we are compelled to consider the dysentery bacilli of all types (fifteen, according to my observations) as constituting a single group of dysentery organisms; in which, however, variations in toxicity, virulence, and even pathological properties between certain strains may exist.

The object of the present paper is to communicate the results of a comparative study of the fermentative properties and the agglutinative and bacteriolytic reactions of the different strains of bacilli which are now generally considered to be the etiological factor in acute dysentery and the allied intestinal conditions. The results of the examination of the virulence, the pathological-anatomical lesions produced, together with the clinical features of the human cases of infection with the different strains, will be considered elsewhere.

STRAINS OF DYSENTERY BACILLI STUDIED.

In order to render my observations as complete as possible, I determined to collect as many different strains of dysentery bacilli as I could. Some of these were obtained from countries other than Japan, namely, from Korea, Manchuria, and the Philippine Islands; others were isolated from patients of the Japanese fleet during the Russo-Japanese war and from those of the Russian Baltic fleet who by capture fell into the hands of the Japanese naval and military surgeons. In all, seventy-four strains were collected. Among these were:

Four strains from this institute, one of the original "Shiga" type and three so-called "acid bacilli" (one isolated from a case of laboratory infection).

Four strains of Manchurian dysentery bacilli, three of the non-acid type, and one of the acid. These were obtained through the kindness of Dr. S. Hata, a lieutenant surgeon of the Japanese military service, who isolated two of the cultures in Rioyang and the other two at Gaiping. The strains were carefully identified by me (24).

Three non-acid bacilli isolated from cases of dysentery among sailors of the Japanese squadron while it was lying in the Yalu Sea.[2] The strains were sent to me by Dr. I. Hirano, fleet surgeon of Imperial Japanese navy.

Two non-acid strains from the Philippines which I owe to the kindness of Dr. Strong, of Manila, who isolated and verified them.

Five strains from Korea, one of the non-acid and the other four of acid types, for which I have to thank Dr. Momose (25), who isolated them in an epidemic among natives at Chinkaiwang.

One non-acid strain from Shizuoka, situated near Mt. Fuzi, which, with Dr. Owada of this institute, I isolated in an epidemic occurring in that place.

Two non-acid strains which Dr. Hara (26) and I isolated from an epidemic in Kanagawa in 1904.

One non-acid strain which I isolated from a case of sporadic dysentery (in 1905) at Ebara, a province of Tokio perfecture.

Two strains from Port Arthur (non-acid type) for which I owe my thanks to my colleague Dr. Oshida, surgeon of the Imperial Japanese army, who isolated them from cases of dysentery contracted in the army before Port Arthur.

Six acid strains obtained from Russian patients on board the Russian Baltic fleet after their capture. For these I am indebted to Dr. Oshida (27) who, after the siege of Port Arthur, investigated the subject of dysentery at Ninoshima Quarantine Station.

Three strains from Wakayama, one of the non-acid type and two others of the acid type, which I isolated in an epidemic of dysentery at Wakayama, near Osaka, in 1905.

Forty-one strains of dysentery bacilli, all of the acid type, which I isolated (28) in the last epidemic in Tokio (July, 1904–August, 1905.) Most of these were isolated from very severe cases of acute dysentery, but some of them were obtained from cases of so-called winter diarrhœa, in 1905.[3]

[2] This was the only epidemic of dysentery in our navy during the Russo-Japanese war.

[3] Five cases of the latter affection, one of which died and upon which an autopsy was performed, were reported by me (29) and the organism isolated was described as a new type of dysentery bacillus. This was placed in an intermediate position between the acid and non-acid types. These observations show the incorrectness of the distinctions of the two types proposed by Lentz.

One strain from Kyoto of the acid type which was isolated by Dr. Yoshida (30), who regarded it as a variety of the dysentery bacillus (Shiga). The organism was identified by me.

A comparison of all these cultures revealed the fact that they could not be distinguished one from another, either culturally or morphologically, although *minor* differences were shown to exist, particular in their characteristics in artificial culture media.

In addition to the agglutination test, the action of bacteriolytic immune serum upon all the strains was studied, since this is perhaps the most important test for the differentiation and identification of bacteria. This was performed *in vitro,* as the virulence of the different strains varied greatly.

TYPES AS DETERMINED BY FERMENTATION.

All the strains of dysentery bacilli mentioned above have been cultivated for many generations on artificial media (common agar). In many instances in which the organisms were isolated by myself, the first fermentation tests were made immediately upon isolation; in others this was impossible, as the organisms were sent to me subsequent to their isolation. Multiple tests as proposed by Hiss have been made, and the final tests of all the strains were performed more than a year after their isolation, because my investigations on this subject continued for about one year. Whenever unexpected reactions or appearances were manifested, fresh plate cultures were made to determine the purity of the original culture and the experiments were repeated with the various colonies. The same results were invariably obtained. Dextrose, maltose, saccharose, dextrin, lactose, and alcohol-mannite were the fermentable substances used. The tests with galactose, lævulose, and inulin were omitted since, according to Hiss, no differences of value as distinguished from those obtained with dextrose, were shown to exist on cultivation in this medium, and none of the bacilli fermented inulin with acid production.

The common peptone-water solution was used as a nutrient medium, to which the carbohydrates were added in sufficient quantity to form a 1.3 per cent solution, as suggested by Lentz. Merck's preparation of litmus, highly purified, was used in all the experiments and was added in sufficient amount to form a 0.6 per cent solution. The peptone medium was composed of 0.5 per cent sodium chloride and 1 per cent peptone [sic.] in 100 cubic centimeters hydrant water; it was sterilized in the usual manner and its reaction tested and, if necessary, it was neutralized. Sugar was then added and the medium placed in tubes and sterilized for from ten to fifteen minutes on three consecutive days. The color of the medium is of a light blue. A sufficient acid production changes the medium to a red color; when acid is formed to a less extent, the color is altered to purple. The variations in composition of the nutrient media to which the sugars were added, and the variations in the salts present, which Hiss undertook were not performed by me

because of the slight differences which Hiss found. All the observations were made at a temperature of 37° C. and were continued for fifteen days.

Table I shows the results of the comparative fermentation tests of the fifteen types of dysentery bacilli with five carbohydrates and with alcohol mannite during fifteen days. The results of the tests made with the other strains are omitted because they showed no variations in fermentation differing from those of the fifteen types enumerated. Table II gives the same results expressed in a somewhat simpler form.

TABLE I.

Type.	Name.	Dextrose.						Mannite.						Maltose.					
		1	3	5	7	10	15	1	3	5	7	10	15	1	3	5	7	10	15
A	Shiga	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
B	Port Arthur	+	+	+	+	+	+	−	−	−	−	−	−	−	±	+	+	+	+
C	Yalu	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
D	Rio-Yang II	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
E	Korea I	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
F	Wakayama I	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
G	Tokyo I	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
H	Korea II	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
I	Rio-Yang I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
J	Baltic II	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
K	Tokyo III	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
L	Yamaguchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
M	Tokyo IV	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N	Korea III	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
O	Kyoto	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Type.	Name.	Saccharose.						Dextrin.						Lactose.					
		1	3	5	7	10	15	1	3	5	7	10	15	1	3	5	7	10	15
A	Shiga	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
B	Port Arthur	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
C	Yalu	−	−	+	+	+	±	−	−	−	−	−	−	−	−	−	−	−	−
D	Rio-Yang II	−	−	−	−	−	−	−	−	±?	+	+	+	−	−	−	−	−	−
E	Korea I	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	±	±	±
F	Wakayama I	−	−	?	+	+	+	+	+	+	+	+	+	±	±	+	+	+	+
G	Tokyo I	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
H	Korea II	−	±	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
I	Rio-Yang I	±	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
J	Baltic II	−	−	−	−	−	−	−	−	−	±	+	+	−	−	−	−	−	−
K	Tokyo III	−	−	+	+	+	+	−	−	+	±	+	+	−	−	−	−	−	−
L	Yamaguchi	+	+	+	+	+	+	−	+	+	±	±	±	−	−	−	+	+	+
M	Tokyo IV	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
N	Korea III	−	±	+	+	+	+	−	−	+	+	+	+	−	±	+	+	+	+
O	Kyoto	−	−	+	±	+	+	−	−	+	+	+	+	−	−	±	±	±	+

TABLE II.—*The types of dysentery bacilli showing the results in fermentation after fifteen days.*

Type.	Name.	Dextrose.	Mannite.	Maltose.	Saccharose.	Dextrin.	Lactose.
A	Shiga	+	—	—	—	—	—
B	Port Arthur	+	—	+	—	—	—
C	Yalu	+	—	—	+	—	—
D	Rio-yang II	+	—	+	—	+	—
E	Korea I	+	—	—	—	—	+
F	Wakayama I	+	—	—	+	+	+
G	Tokyo I	+	+	—	—	—	—
H	Korea II	+	+	—	+	—	—
I	Rio-yang I	+	+	+	+	—	—
J	Baltic II	+	+	—	—	+	—
K	Tokyo III	+	+	—	+	+	—
L	Yamaguchi	+	+	+	+	+	—
M	Tokyo IV	+	+	+	—	—	+
N	Korea III	+	+	—	+	+	+
O	Kyoto	+	+	+	+	+	+

Practically the same results were obtained after a period of seven days, but after this time not so much variation in fermentation takes place. Hiss divided dysentery bacilli into four types, basing his classification upon the results of his fermentation tests after five days. If, from the results of my observations upon fermentation after five days, I should attempt to separate the types of dysentery bacilli, it would be necessary to recognize at least thirteen of these, as Table III shows. However, this would obviously be incorrect because of the incompleteness of the observations.

TABLE III.—*Showing the results of fermentation tests made upon the fifteen types of dysentery bacilli after five days.*

Type.	Dextrose.	Mannite.	Maltose.	Saccharose.	Dextrin.	Lactose.
A and D	+	—	—	—	—	—
B	+	—	+	—	—	—
C	+	—	—	+	—	—
E	+	—	—	—	—	+
F	+	—	—	—	+	+
G and J	+	+	—	—	—	—
H	+	+	—	+	—	—
I	+	+	+	+	—	—
K	+	+	—	+	+	—
L	+	+	+	+	+	—
M	+	+	+	—	—	—
N	+	+	—	+	+	+
O	+	+	+	—	+	—

The above results may be summarized as follows:

The organisms studied fall into fifteen groups by reason of certain differences in their power to ferment carbohydrates and alcohol mannite.

The *first group*, or "A" type, is represented by the "Shiga," which ferments only dextrose; the *second*, or "B" type, by the culture "Port Arthur," which was isolated by Dr. Oshida from Russian military officers suffering from acute epidemic dysentery. It attacks maltose in addition to dextrose, producing acid.

The *third*, or "C" type, is represented by the culture which I have called "Yalu." It was isolated from a few dysentery patients among sailors of the Japanese squadron cruising on the Yalu Sea. The type ferments saccharose in addition to dextrose.

The *fourth*, or "D" type, is the culture "Rio-yang II," which was isolated after the siege of Rio-yang from a patient near that place in Manchuria. It is capable of fermenting both dextrose and dextrin.

The *fifth*, or "E" type, is designated as "Korea I" and ferments both dextrose and lactose. It was isolated by naval fleet surgeon Dr. Momose from a patient at Chin-Kai-wang, Korea.

The *sixth*, or "F" type, is represented by the culture "Wakayama I," which I isolated in an epidemic at Wakayama (situated about 20 miles distant from Osaka). The organism attacks saccharose, dextrin, and lactose in addition to dextrose.

The *seventh*, or "G" type, is represented by the culture "Tokyo I," which ferments dextrose and alcohol-mannite. This type was first isolated by Flexner, and afterwards was classified by Hiss as Type II and by Park, Collins, and Goodwin as Type III. The strain with which my experiments were made was obtained in Japan by Kawaguchi and Tsuchiya, but at the time of its isolation it was thought to be identical with the "Shiga" type. Later I studied its exact characters and afterwards Dr. Futaki (31) (1903), Nakajo (32), and Tsuchiya (33) (1904) also reported upon them. This type is identical with the "Y" type of Hiss and Russel.

The *eighth*, or "H" type, is represented by "Korea II," which differs from the type "G" in fermenting saccharose. This organism is identical with the "Strong" type of Hiss (first isolated by Strong).

The *ninth*, or "I" type, is illustrated by the culture "Rio-yang II," which, besides dextrose, also ferments mannite, maltose, and saccharose.

The *tenth*, or "J" type, is represented by the culture "Baltic II." This organism ferments not only dextrose and mannite, but also dextrin. This type was studied by military surgeon Dr. Oshida; it was isolated from captured Russian naval officers suffering from dysentery on board the Baltic fleet after the battle of the Japanese Sea and was sent by him to me.

I am justified in considering it as a strain having a different source from those of the same type isolated in Japan, since the Russian patient from which it was obtained had not been in Japan nor in communication with that country.

The *eleventh*, or "K" type, is represented by the culture "Tokyo III," which differs from type "J" only by the fact that it ferments saccharose.

The *twelfth*, or "L" type, is represented by the culture "Yamaguchi," which differs from the "O" type only in not fermenting lactose.

The *thirteenth*, or "M" type, is represented by the culture "Tokyo IV," which, besides dextrose and mannite, also ferments maltose and lactose. The fermentation, except in the case of lactose, is usually rapid, being distinctly manifest in twenty-four to forty-eight hours.

The *fourteenth*, or "N" type, is represented by the culture "Korea III," which attacks dextrose, saccharose, dextrin-lactose, and alcohol-mannite, while it does not ferment maltose.

The *fifteenth*, or "O" group, is that represented by "Kyoto" as the type. As we have seen, this organism has the ability, besides mannite, also to ferment dextrose, maltose, saccharose, dextrin, and even lactose. The organism is the same as that of "Duval," group of Duval (34).

These fifteen types of dysentery bacilli may again be divided into two major groups according to their different behavior in regard to the fermentation of alcohol-mannite, the members of one not fermenting and those of the other attacking mannite with the production of acid. The first major group (so-called non-acid bacilli) comprises organisms corresponding to the ones described by Shiga-Kruse as the cause of epidemic dysentery. Types "A," "B," "C," "D," "E," and "F," which I have studied, belong in this group. Among them only the "A" and "B" types were carefully identified by previous authors, whereas the other four have been recognized by myself. The second major group (so-called acid bacilli), separated from the first by their power to ferment alcohol-mannite, is composed of organisms which may again be separated into nine groups by their different action in fermenting other carbohydrates. Among the nine groups which ferment mannite only, four have been found in Europe and America. These are "G" type ("Y" type of Hiss), "H" type (Strong's type of Hiss), "L" type (Harris type of Hiss), and "O" type ("Duval" type of Duval), while the other five have been identified by me in different strains obtained in Japan, Korea, Manchuria, and from the Baltic fleet.

However, this method of division, into non-acid and acid types of the bacilli according to their power of fermenting alcohol-mannite, can not be considered to be a correct one. As we have seen, even among the two major types all the strains differ from each other in their power to ferment the different carbohydrates, but these characteristics are not sufficiently regular to enable us always to divide them distinctly. On the other hand, moreover, when we come to study the agglutination and bacteriolytic reactions of the different strains, we find that a division into similar groups is not justifiable. Arguing from my observations, I can see no reason for the separation of the acid and non-acid bacilli into two distinct groups as proposed by Lentz, and no justification for designating any bacillus among the group of organisms which causes dysentery as a "pseudo-dysentery" bacillus as proposed by Kruse. It seems to me that we are rather compelled to consider all fifteen types of the dysentery bacilli as comprising a single group and constituting the whole of this group of organisms and to admit that minor distinctions which sometimes irregularly occur may exist between the different strains.

As soon as I had determined, by fermentation tests, that the fermentative properties of the fifteen types of dysentery bacilli were not of such a nature as to allow us always to separate them distinctly and that their separation, based upon their action upon alcohol-mannite, did not always conform to their division into the same groups if founded upon their agglutinative reactions, I undertook a careful comparative study of the agglutination reactions of all of these cultures toward animal immune serum.

In most instances, although tests were made with the other strains, only the reactions obtained with the fifteen already referred to and tabulated will be given in the tables, excepting in particular instances. Rabbits in most cases were used for the preparation of the serum since they are the most convenient laboratory animals for immunization and because, according to the German authors, these animals do not produce secondary agglutinins, and therefore their serum is best suited for the differentiation of these organisms. Goats and horses were employed in only a few instances.

As the organisms fermenting mannite are generally not very pathogenic for these animals, their immunization is a simple matter. Only two or three strains of the same type were used for immunization. The so-called non-acid types were as a rule toxic for rabbits and, unless great care was taken, the animals died from the introduction of either the dead or the living cultures, but among these types of bacilli, some strains were encountered which were not so toxic, even when they had but recently been isolated. The most satisfactory results in the production of sera were obtained from the administration of very small, primary, subcutaneous doses of living cultures, as advised by Hiss, allowing full recovery of the animals to take place before a second injection of an increased dose was given.

The technique employed in the agglutination experiments consisted of the macroscopic test carried on in small test tubes.

Suspensions of agar cultures, eighteen to twenty hours old, were made in 0.85 per cent of sodium chloride solution, 8 cubic centimeters of saline solution being always employed for 8 milligrams of agar culture. The quantity of suspension of the cultures and serum in the tubes was always made up to 2 cubic centimeters, 0.5 cubic centimeter of suspension (that is, 0.5 milligram of agar culture) being employed. Twenty-four hours was the limit of the test. The results determined by the use of the macroscopic method were as follows (see Tables IV, V, VI):

TABLE IV.—*Agglutination with serum from a rabbit immunized against "Tokyo I" strain, "G" type (acid on mannite).*

Type.	Organism.	Mannite group.	Serum dilutions. 1-20.	1-40.	1-75.	1-100.	1-200.	1-300.	1-500.	1-750.	1-1,000.	1-2,000.	1-3,000.	1-5,000.	1-7,500.	1-10,000.	Limit of agglutination.
A	Shiga	Non-acid	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—(1-20)
B	Port Arthur	Non-acid	+	—	—	—	—	—	—	—	—	—	—	—	—	—	1-20
C	Yalu	Non-acid	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—(1-20)
D	Rio-yang II	Non-acid	+	+	+	—	—	—	—	—	—	—	—	—	—	—	1-75
E	Korea I	Non-acid	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—(1-20)
F	Wakayama	Non-acid	+	—	—	—	—	—	—	—	—	—	—	—	—	—	1-20
G	Tokyo I	Acid	+	+	+	+	+	+	+	—	—	—	—	—	—	—	1-500
H	Korea II	Acid	+	+	+	+	+	+	+	—	—	—	—	—	—	—	1-500
I	Rio-yang I	Acid	+	+	+	—	—	—	—	—	—	—	—	—	—	—	1-75
J	Baltic II	Acid	+	+	+	+	+	+	+	+	—	—	—	—	—	—	1-750
K	Tokyo III	Acid	+	+	—	—	—	—	—	—	—	—	—	—	—	—	1-40
L	Yamaguchi	Acid	+	+	+	+	+	+	+	+	+	—	—	—	—	—	1-1,000
M	Tokyo IV	Acid	+	+	+	+	+	+	+	+	—	—	—	—	—	—	1-750
N	Korea III	Acid	+	+	+	+	+	+	+	+	—	—	—	—	—	—	1-750
O	Kyoto	Acid	+	+	+	+	+	+	+	+	+	—	—	—	—	—	1-1,000

TABLE V.—*Agglutination with serum from a rabbit immunized against "Rio-yang I," "I" type (acid on mannite).*

Type.	Organism.	Mannite group.	Serum dilutions. 1-20.	1-40.	1-75.	1-100.	1-200.	1-300.	1-500.	1-750.	1-1,000.	1-2,000.	1-3,000.	1-5,000.	1-7,500.	1-10,000.	Limit of agglutination.
A	Shiga	Non-acid	+	+	—	—	—	—	—	—	—	—	—	—	—	—	1-40
B	Port Arthur	Non-acid	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—(1-20)
C	Yalu	Non-acid	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—(1-20)
D	Rio-yang II	Non-acid	+	—	—	—	—	—	—	—	—	—	—	—	—	—	1-20
E	Korea I	Non-acid	+	+	—	—	—	—	—	—	—	—	—	—	—	—	1-40
F	Wakayama I	Non-acid	+	+	—	—	—	—	—	—	—	—	—	—	—	—	1-40
G	Tokyo I	Acid	+	+	+	+	—	—	—	—	—	—	—	—	—	—	1-100
H	Korea II	Acid	+	+	+	+	+	+	+	—	—	—	—	—	—	—	1-500
I	Rio-yang I	Acid	+	+	+	+	—	—	—	—	—	—	—	—	—	—	1-100
J	Baltic II	Acid	+	+	—	—	—	—	—	—	—	—	—	—	—	—	1-40
K	Tokyo III	Acid	+	+	+	+	+	+	—	—	—	—	—	—	—	—	1-300
L	Yamaguchi	Acid	+	+	+	+	+	—	—	—	—	—	—	—	—	—	1-200
M	Tokyo IV	Acid	+	—	—	—	—	—	—	—	—	—	—	—	—	—	1-20
N	Korea III	Acid	+	+	+	+	+	—	—	—	—	—	—	—	—	—	1-200
O	Kyoto	Acid	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—(1-20)

TABLE VI.—*Agglutination of the fifteen types of dysentery bacilli with sera of type "O," "G," "L" (rabbit, goat, and horse sera).*

Type.	Organism.	Antidysenteric serum.				
		"Kyoto" (rabbit).	"Tokyo I" (goat).	"Yamaguchi" (goat).	"Tokyo I" (horse).	Mannite fermentation.
A	Shiga	1–40	1–40	1–40	—(1–20)	Non-acid.
B	Port Arthur	—(1–20)	1–20	1–20	—(1–20)	
C	Yalu	—(1–20)	1–40	1–75	—(1–20)	
D	Rio-yang II	1–20	1–100	1–40	1–100	
E	Korea I	—(1–20)	1–75	1–40	—(1–20)	
F	Wakayama I	—(1–20)	1–20	1–40	—(1–20)	
G	Tokyo I	1–75	1–5,000	1–300	1–2,000	Acid.
H	Korea II	1–500	1–500	1–75	1–1,000	
I	Rio-yang I	1–40	1–750	1–100	1–500	
J	Baltic II	1–75	1–2,000	1–200	1–2,000	
K	Tokyo III	1–40	1–200	1–40	1–750	
L	Yamaguchi	1–300	1–500	1–300	1–300	
M	Tokyo IV	1–100	1–2,000	1–300	1–1,000	
N	Korea III	1–300	1–1,000	1–500	1–1,000	
O	Kyoto	1–1,000	—(1–20)	1–300	1–4,000	

Upon examining the results of the agglutination tests tabulated above, we see that in all cases we apparently find a distinction between the strains which ferment mannite and those which do not. This is the reason why most authors have agreed to Lentz's and Martini's proposal to distinguish between the acid and non-acid types according to their powers of fermenting mannite. While in some instances my results are in accord with those of Lentz—i. e., in those sera which were gained by the immunization of rabbits, goats, and even horses, against strains "D," "G," "H," "I," "L," and "O," some of which are described in the above tables (Tables IV, V, and VI), in most instances my results are opposed to his views; particularly is this true with the sera which I have obtained by the immunization of animals against the other types of the bacilli, as the following table shows:

TABLE VII.—*Agglutination of the fifteen types of dysentery bacilli with dysenteric immune serum prepared from several strains.*

Type.	Organism.	Mannite fermentation.	Rabbits' immune serum.			Goats' immune serum.		Horse immune serum "Shiga."
			"Port Arthur."	"Korea III."	"Tokyo IV."	"Shiga."	"Rio-yang I."	
A	Shiga	Nonacid	1–600	—(1–20)	—(1–20)	1–2,000	1–500	1–1,000
B	Port Arthur		1–400	—(1–20)	1–20	1–1,000	1–500	1–1,000
C	Yalu		1–600	1–20	1–40	1–500	1–750	1–1,000
D	Rio-yang II		1–1,000	1–500	1–5,000	1–3,000	1–200	1–6,000
E	Korea I		1–1,000	—(1–20)	1–40	1–750	1–1,000	1–1,000
F	Wakayama I		1–600	—(1–20)	1–2,000	1–2,000	1–1,000	1–1,000

TABLE VII.—*Agglutination of the fifteen types of dysentery bacilli with dysenteric immune serum prepared from several strains*—Continued.

Type.	Organism.	Mannite fermentation.	Rabbits' immune serum.			Goats' immune serum.		Horse immune serum "Shiga."
			"Port Arthur."	"Korea III."	"Tokyo IV."	"Shiga."	"Rio-yang I."	
G	Tokyo I	Acid	1–80	1–500	1–7,500	1–750	1–750	1–300
H	Korea II		1–600	1–2,000	1–1,000	1–1,000	1–2,000	1–2,000
I	Rio-yang I		1–80	1–300	1–750	1–3,000	1–5,000	1–1,000
J	Baltic II		1–200	1–300	1–5,000	1–100	1–300	1–500
K	Tokyo III		1–100	1–2,000	1–750	1–200	1–1,000	1–2,000
L	Yamaguchi		1–200	1–2,000	1–750	1–750	1–5,000	1–2,000
M	Tokyo IV		1–20	1–300	1–5,000	1–500	1–750	1–500
N	Korea III		1–200	1–2,000	1–500	1–2,000	1–5,000	1–2,000
O	Kyoto		1–100	1–1,000	1–2,000	1–7,500	1–100	1–2,000

In Table VII we see the high agglutination of "Korea II" (an acid strain) produced by dysenteric immune serum prepared with "Port Arthur" culture (a non-acid strain). *Vice versa* a similar result in agglutination was obtained with dysenteric immune serum prepared with "Korea III" (an acid strain) on "Rio-yang II" (a non-acid strain). It may also be seen that immune serum "Tokyo IV" (an acid strain) has the power to agglutinate "Rio-yang II" and "Wakayama I" (non-acid strains) in very high dilutions even with dysenteric-immune rabbit serum, which is designated as a "differentiating serum" by German authors. With the immune serum of goats and of horses, as Shiga and some German authors have reported, there is very little difference in the agglutinative reactions between both the acid and the non-acid types. In addition, a study of Table VII will reveal the results of the agglutination tests of all fifteen of the strains of dysentery bacilli with immune serum of rabbits, goats, and horses; by a glance it may at once be seen, that there is no apparent distinction in agglutinative reactions between the so-called mannite fermenting and non-fermenting organisms and that no such distinction can correctly be made.

STUDIES ON BACTERIOLYSIS.

Bacteriolysis by immune serum being an important method in the identification and differentiation of bacterial species, it seems strange to me that most authors who have studied the differentiation of the types

of dysentery bacilli have made no use of it. However, Gay has employed this method in his study of the types of dysentery bacilli. He found that bacteriolytic differences existed which seemed to distinguish the two types, recognized at that time as sharply as the other tests hitherto employed. But, unfortunately, the bacilli which were obtained by him were of just those types which were in a condition to give him reactions suited to these distinctions. If he had been able to collect and study many strains of dysentery bacilli from other sources, he would have discovered that there were no constant agglutinative and bactericidal reactions by means of which the two types could be distinguished. As has already been mentioned, in my researches a great number of strains of dysentery bacilli from different sources were studied. Having found that the agglutinative tests did not bear out the classification of the acid and non-acid bacilli into two distinct groups of organisms, I determined to study the bacteriolytic reactions of all these cultures with the serum of immunized animals. As has already been mentioned in the discussion of the agglutinative reactions, in the study of the bacteriolytic reactions tests were made with other cultures besides the fifteen strains already referred to. However, only the reactions with the latter strains have been tabulated.

As Shiga has already shown, the bacteriolytic power of antidysenteric serum can best be demonstrated *in vitro* by the method devised by Neisser and Wechsberg. The authors found that a fixed amount of normal serum containing complement, of itself incapable of causing bacteriolysis, will destroy a given amount, by weight, of a specific organism in the presence of a definite quantity of complement-free immune serum (as Table VIII shows). The addition of an amount of immue serum greater or less than that exactly requisite, will allow of more or less unrestricted growth of the organisms. The growth obtained when the quantity of immune serum is too small is explained upon the basis of an insufficiency of bacteriolytic amboceptors, and where there is a surplus of immune serum, upon the basis of an excess of amboceptors, through which the complement becomes deviated from the bacterial cells by union with the free amboceptors.

As Tables VIII and IX show, each type of bacillus is bacteriolyzed under the conditions given above with the inactive antidysenteric serum, plus fresh guinea pigs' and horses' serum, respectively, containing complement.

TABLE VIII.—*Bacteriolysis of "Gaiping I" ("A" type) with serum from horse, "Shiga" ("A" type).*

0.002 milligram fresh agar culture "Shiga" bacillus in 1 cubic centimeter salt solution.	"Shiga" dysenteric immune serum, complement free.	Fresh normal guinea pig serum.	Plate colonies after five hours.
1 c. c	None.	None.	Innumerable.
1 c. c	0.1 c. c.	None.	Thousands.
1 c. c	None.	0.2 c. c.	Innumerable.
1 c. c	0.1 c. c.	0.2 c. c.	Do.
1 c. c	0.05 c. c.	0.2 c. c.	Do.
1 c. c	0.025 c. c.	0.2 c. c.	Thousands.
1 c. c	0.01 c. c.	0.2 c. c.	One thousand.
1 c. c	0.005 c. c.	0.2 c. c.	Hundreds.
1 c. c	0.0025 c. c.	0.2 c. c.	Do.
1 c. c	0.001 c. c.	0.2 c. c.	One hundred.
1 c. c	0.0005 c. c.	0.2 c. c.	Almost none.
1 c. c	0.00025 c.c.	0.2 c. c.	Few.
1 c. c	0.0001 c. c.	0.2 c. c.	Hundreds.
Control, no bacilli	None.	0.2 c. c.	None.
Control, no bacilli	0.1 c. c.	None.	Do.

TABLE IX.—*Bacteriolysis of "Rio-yang I" ("I" type) with serum from rabbit, "Tokyo I" ("G" type).*

0.002 milligram fresh agar culture "Rio-yang" bacillus in 1 cubic centimeter salt solution.	"Tokyo I" anti-dysenteric serum, complement free.	Fresh normal horse serum.	Plate colonies after five hours.
1 c. c	None.	None.	Innumerable.
1 c. c	0.1 c. c.	None.	Do.
1 c. c	None.	0.2 c. c.	Thousands.
1 c. c	0.1 c. c.	0.2 c. c.	One thousand.
1 c. c	0.05 c. c.	0.2 c. c.	One hundred.
1 c. c	0.025 c. c.	0.2 c. c.	None.
1 c. c	0.01 c. c.	0.2 c. c.	One hundred.
1 c. c	0.005 c. c.	0.2 c. c.	One thousand.
1 c. c	0.0025 c. c.	0.2 c. c.	Thousands.
1 c. c	0.001 c. c.	0.2 c. c.	Do.
1 c. c	0.0005 c. c.	0.2 c. c.	Innumerable.
1 c. c	0.00025 c.c.	0.2 c. c.	Do.
1 c. c	0.0001 c. c.	0.2 c. c.	Do.
Control, no bacilli	None.	0.2 c. c.	None.
Control, no bacilli	0.1 c. c.	None.	Do.

The results obtained by the use of a fixed amount of homologous immune serum sufficient to bring about the destruction of a definite amount of culture of *B. dysenteriæ* in the presence of a sufficiency of complement are shown by Tables VIII and IX. These are the only

examples of bacilli from Manchuria (Rio-yang and Gai-ping) which I tested with the anti-dysenteric serum of our Government institute.

Experiments in cross bacteriolysis were also performed—that is, the action upon a non-acid strain by an immune serum prepared with an acid strain, or vice versa, was studied. In these experiments I have used only the sera of rabbits and have purposely avoided the employment of those of goats and horses, since it has been stated that these animals sometimes furnish large amounts of secondary agglutinins.

In the tables only the final results are given, since it seemed desirable at the same time to compare them with those of fermentation and agglutination.

TABLE X.—*Bacteriolysis of the fifteen types of dysentery bacilli with rabbit sera immunized against "Tokyo I" ("Y" type, Hiss,) "Rio-yang I," and "Yamaguchi" ("Harris" type).*

Type.	Dysentery bacilli marked according to their source.	Mannite fermentation.	Anti-dysenteric serum, "Tokyo I" (acid). Bacteriolysis. 0–20.	40–75.	500.	750–1,000.	Limit of agglutination.	Anti-dysenteric serum, "Rio-yang I" (acid). Bacteriolysis. 0–40.	100–200.	300–500.	Limit of agglutination.	Anti-dysenteric serum, "Yamaguchi" (acid). Bacteriolysis. 0–40.	75–100.	300–1,000.	Limit of agglutination.
A	Shiga	Non-acid	—	--	--	--	—(1–20)	—	--	--	1–40	—	--	--	—(1–20)
B	Port Arthur	Non-acid	—	--	--	--	1–20	—	--	--	—(1–20)	—	--	--	—(1–20)
C	Yalu	Non-acid	—	--	--	--	—(1–20)	—	--	--	—(1–20)	—	--	--	—(1–20)
D	Rio-yang I	Non-acid	--	—	--	--	1–75	—	--	--	1–20	—	--	--	1–40
E	Korea I	Non-acid	—	--	--	--	—(1–20)	—	--	--	1–40	—	--	--	1–20
F	Wakayama I	Non-acid	—	--	--	--	1–20	—	--	--	1–40	—	--	--	—(1–20)
G	Tokyo I	Acid	--	--	+	--	1–500	--	+	--	1–100	--	+	--	1–100
H	Korea II	Acid	--	--	—	--	1–500	--	--	+	1–500	--	--	+	1–1,000
I	Rio-yang I	Acid	--	+	--	--	1–75	--	+	--	1–100	—	--	--	1–40
J	Baltic II	Acid	--	--	--	+	1–750	+	--	--	1–40	--	+	--	1–75
K	Tokyo III	Acid	--	+	--	--	1–40	--	--	+	1–300	--	—	--	1–100
L	Yamaguchi	Acid	--	--	--	—	1–1,000	--	—	--	1–200	--	--	+	1–1,000
M	Tokyo IV	Acid	--	--	--	—	1–750	+	--	--	1–20	--	+	--	1–75
N	Korea III	Acid	--	--	--	+	1–750	--	+	--	1–200	--	--	+	1–300
O	Kyoto	Acid	--	--	--	—	1–1,000	--	--	--	1–500	--	--	+	1–300

On the examination of the above table, we see that, under the conditions stated, no cross bacteriolysis is obtained between both types. But this is only the case when they are treated with anti-dysenteric serum from "G" type (" Y," Hiss), "I" type, and "L" type ("Harris" type) of dysentery bacilli. If we observe the bacteriolysis with anti-dysenteric serum from bacilli of the other types, we can see that the cross bacteriolysis between both is produced.

TABLE XI.—*Bacteriolysis of the fifteen types of dysentery bacilli with anti-dysenteric horse serum, "Tokio I" and "Shiga."*

Type.	Dysentery bacilli marked according to their source.	Mannite fermentation.	Anti-dysenteric serum, "Tokyo I" (acid).					Anti-dysenteric serum, "Shiga" (non-acid).			
			0.	100–300.	500–1,000.	2,000–4,000.	Limit of agglutination.	300.	500–1,000.	2,000–4,000.	Limit of agglutination.
			Bacteriolysis.					Bacteriolysis.			
A	Shiga	Non-acid	—	--	--	--	—(1–20)	--	+	--	1–1,000
B	Port Arthur	Non-acid	—	--	--	--	—(1–20)	--	+	--	1–1,000
C	Yalu	Non-acid	—	--	--	--	—(1–20)	--	—	--	1–1,000
D	Rio-yang II	Non-acid	--	+	--	--	1–100	--	--	+	1–4,000
E	Korea I	Non-acid	—	--	--	--	—(1–20)	--	+	--	1–1,000
F	Wakayama I	Non-acid	—	--	--	--	—(1–20)	--	—	--	1–1,000
G	Tokyo I	Acid	--	--	--	+	1–2,000	—	--	--	1–300
H	Korea II	Acid	--	--	—	--	1–1,000	--	--	+	1–2,000
I	Rio-yang I	Acid	--	--	+	--	1–500	--	—	--	1–1,000
J	Baltic II	Acid	--	--	--	+	1–2,000	--	—	--	1–500
K	Tokyo III	Acid	--	--	+	--	1–750	--	--	+	1–2,000
L	Yamaguchi	Acid	--	+	--	--	1–300	--	—	--	1–1,000
M	Tokio IV	Acid	--	--	+	--	1–1,000	--	—	--	1–500
N	Korea III	Acid	--	--	+	--	1–500	--	--	—	1–2,000
O	Kyoto	Acid	--	--	--	—	1–1,000	--	--	—	1–2,000

The last table, XI, shows that with anti-dysenteric horse serum the evidences of "cross bacteriolysis" between both types can also be obtained. If we omit the results with the "D" type (Rio-yang II, non-acid) we should be led to the wrong conclusion, namely, that cross bacteriolysis did not exist in the bacteriolysis with the anti-dysenteric horse serum of the "G" type (Tokyo I); this would also be the case with the horse serum from "A" type (Shiga), and if, in these tests, we had not the results with "H" (Korea II) and "K" (Tokyo III) we would arrive at the same faulty conclusion. It therefore is apparent why bacteriolytic tests have frequently led various authors to conform to the opinions of Lentz. In Table XI we can also see the nonconformity of the fermentation and agglutination tests with the bacteriolytic reactions. We can observe the positive bacteriolysis of the non-acid type "D" (Rio-yang II) with anti-dysenteric serum from the acid type and the negative reaction of types "C" (Yalu) and "F" (Wakayama I) with the homologous—i. e., non-acid—anti-dysenteric serum from "A" (Shiga). A further study of Table XII will reveal a number of other cross bacteriolytic reactions and also many negative ones with the homologous anti-dysenteric sera. Among the latter we will also find that the "A" (Shiga) type shows no evidence of bacteriolysis by the anti-dysenteric rabbit serum "B" (Port Arthur).

TABLE XII.—*Bacteriolysis of fifteen types of dysentery bacilli with rabbit sera immunized against "Port Arthur," "Tokyo IV," "Baltic II," and "Kyoto."*

Type.	Dysentery bacilli named according to their source.	Mannite fermentation.	Anti-dysenteric serum, "Port Arthur" (non-acid). Bacteriolysis. 20.	80–100.	200–400.	600–1,000.	Limit of agglutination.	Anti-dysenteric serum, "Tokyo IV" (acid). Bacteriolysis. 0.	20–40.	75–100.	200–300.	Limit of agglutination.
A	Shiga	Non-acid	--	--	--	—	1–600	—	--	--	--	—(1-20)
B	Port Arthur		--	--	+	--	1–400	+	--	--	--	—(1-20)
C	Yalu		--	--	--	+	1–600	—	--	--	--	—(1-20)
D	Rio-yang II		--	--	--	+	1–1,000	+	--	--	--	—(1-20)
E	Korea I		--	--	--	+	1–1,000	--	—	--	--	1-20
F	Wakayama I		--	--	+	--	1–400	—	--	--	--	—(1-20)
G	Tokyo I	Acid	--	+	--	--	1–80	--	--	--	—	1–200
H	Korea II		--	--	--	+	1–600	--	--	+	--	1–100
I	Rio-yang I		--	—	--	--	1–80	--	--	--	--	1–75
J	Baltic II		--	--	—	--	1–200	--	--	--	+	1–300
K	Tokyo III		--	—	--	--	1–100	--	+	--	--	1–40
L	Yamaguchi		--	--	—	--	1–200	--	--	+	--	1–100
M	Tokyo IV		—	--	--	--	1–20	+	--	--	+	1–300
N	Korea III		--	--	—	--	1–200	--	--	+	--	1–100
O	Kyoto		--	—	--	--	1–100	--	--	--	--	—(1-20)

Type.	Dysentery bacilli named according to their source.	Mannite fermentation.	Anti-dysenteric serum, "Baltic II" (acid). Bacteriolysis. 0–40.	500–700.	1,000–2,000.	5,000–7,500.	Limit of agglutination.	Anti-dysenteric serum, "Kyoto" (acid). Bacteriolysis. 0–40.	75–100.	300–1,000.	Limit of agglutination.
A	Shiga	Non-acid	—	--	--	--	—(1–20)	+	--	--	1–40
B	Port Arthur		—	--	--	--	1–20	—	--	--	—(1-20)
C	Yalu		—	--	--	--	1–40	—	--	--	—(1-20)
D	Rio-yang II		--	--	--	+	1–5,000	+	--	--	1–20
E	Korea I		—	--	--		1–40	—	--		—(1-20)
F	Wakayama I		--	--	—	--	1–2,000	—	--	--	—(1-20)
G	Tokyo I	Acid	--	--	--	+	1–7,500	--	—	--	1–75
H	Korea II		--	--	+	--	1–1,000	--	--	+	1–500
I	Rio-yang I		--	+	--	--	1–750	—	--	--	1–40
J	Baltic II		--	--	--	+	1–5,000	--	+	--	1–75
K	Tokyo III		--	—	--	--	1–750	—	--	--	1–40
L	Yamaguchi		--	+	--	--	1–750	--	--	+	1–300
M	Tokyo IV		--	--	--	—	1–5,000	--	—	--	1–100
N	Korea III		--	—	--	--	1–500	--	--	+	1–300
O	Kyoto		--	--	+	--	1–2,000	--	--	+	1–1,000

From all these experiments we can conclude that bacteriolysis of the different strains *in vitro* may take place irrespective of the type, whether acid or non-acid, and also irrespective of whether the immune serum has been prepared with an acid or non-acid strain. On the basis of these facts, and in view of the results of the fermentation and agglutination tests, I must again emphasize that I can see no reason whatever for recognizing two distinct groups of bacilli as suggested by Lentz, namely, those which ferment mannite and those which do not, nor for distinguishing between the so-called "dysentery" and "pseudo-dysentery" bacilli as proposed by Kruse. Furthermore, these results are in conformity with those of Flexner, Strong, Gay, and other American authors, in showing that both types of dysentery bacilli have equal etiological importance and that the differences between them are unimportant.

In the preceding pages I have demonstrated that there are fifteen varieties of dysentery bacilli, nine of which ferment mannite and six of which do not. I have also proved that the separation of these varieties into two groups according to their power of fermenting mannite can not be made in conformity with their agglutinative and bacteriolytic reactions, and, in addition, I have endeavored to test the comparative agglutinative and bacteriolytic reactions of bacilli which have the same properties in regard to fermentation. Among the seventy-four strains of dysentery bacilli which have been isolated in Japan, Korea, Manchuria, from the Japanese and Baltic fleets, and in the Philippine Islands, four are of the "A" type ("Shiga"), six of the "L" type ("Yamaguchi"), and seven of the "I" type ("Rio-yang I"). As Tables XIII, XIV, and XV show, the several strains of the same type do not possess homologous agglutinative and bacteriolytic reactions against the homologous antidysenteric serum.

TABLE XIII.—*Agglutination and bacteriolysis of bacilli of the same type with "Shiga" ("A") with the same sera.*

Bacilli tested.	"Port Arthur II."	"Gaiping I."	"Shiga."	"Philippine I."
Anti-dysenteric serum (rabbit serum):				
"Shiga" ("A" type, non-acid)—				
Agglutination	600	600	1,000	600
Bacteriolysis	—	0.005	0.001	—
"Kyoto" ("O" type, acid)—				
Agglutination	—(1–20)	—(1–20)	75	200
Bacteriolysis	0.01	0.005	0.001	—

TABLE XIV.—*Agglutination and bacteriolysis of bacilli of the same type with "Yamaguchi" ("L" type), with sera "B" and "O."*

Bacilli tested.	"Arisaka."	"Kasuyo."	"Okabe."	"Toyama."	"Yamaguchi."	"Odomasa."
Anti-dysenteric serum (rabbit serum):						
"B" (non-acid)—						
Agglutination	200	—(1–20)	—(1–20)	200	—(1–20)	—(1–20)
Bacteriolysis	—	0.0025	—	0.01	0.05	0.0005
"O" (acid)—						
Agglutination	300	—(1–20)	—(1–20)	20	20	—(0–20)
Bacteriolysis	0.005	—	0.005	0.0005	0.001	0.005

TABLE XV.—*Agulutination and bacteriolysis of bacilli in the same type with "Rio-yang I" ("I" type), with sera "B" and "O."*

Bacilli tested.	"Ito."	"Iijina."	"Morishita."	"Miyake."	"Baltic I."	"Ota."	"Rio-yang I."
Anti-dysenteric serum (rabbit serum):							
"B" (non-acid)—							
Agglutination	40	80	80	80	40	—(1–20)	80
Bacteriolysis	—	0.01	0.0005	0.0025	0.025	0.025	—
"O" (acid)—							
Agglutination			200	40	—(1–20)	20	
Bacteriolysis			—	—	0.01	—	

It is interesting to compare the reaction of "Port Arthur II" and "Philippine I" strains (see Table XIII). These organisms have the same properties in regard to fermentation as has the "Shiga" strain ("A" type), but they do not react with positive bacteriolysis to anti-dysenteric serum from the "A" type (Shiga). On the other hand, the "Port Arthur II" and "Gai-ping I," which behave like the "Shiga" ("A" type) in respect to fermentation, react with positive bacteriolysis to anti-dysenteric serum against "Kyoto" strain ("O" type). In addition, the "Philippine I" strain shows a high agglutination (1–200) but the "Shiga" only a low one (1–75) to this same serum. However, the Shiga organism shows a higher degree of bacteriolysis (0.001). This same phenomenon is not rare among the strains of two of the other types ("L" and "I"), as Tables XIV and XV show.

Tables XVI and XVII demonstrate that the same phenomena are obtained among selected strains of the same type, which show the same fermentative reactions during five days, and from these experiments we can conclude that even among strains of the same type in fermentation

there is no conformity between the agglutinative and bacteriolytic reactions. It is also theoretically unreasonable to suppose that all three receptors (fermentative, agglutinative, and bacteriolytic in the bacillus), which are entirely different in their nature, must always be exactly in conformity with each other in one bacterial cell.

TABLE XVI.—*Agglutination and bacteriolysis of bacilli which are of the same type as "A" (Shiga) in fermentation reactions during five days, with the same sera.*

Bacilli tested.	"Port Arthur II."	"Shizuoka."	"Port Arthur."	"Shiga."	"Philippine I."	"Tokyo X."
Anti-dysenteric serum (rabbit serum):						
"Port Arthur" ("B" type, non-acid)—						
Agglutination	2,000	1,000	400	600	1,000	600
Bacteriolysis	0.001	0.0025	0.0005	—	—	0.0001
"Kyoto" ("O" type, acid)—						
Agglutination	—(1-20)	—(1-20)	—(1-20)	40	—(1-20)	20
Bacteriolysis	0.05	0.005	—	0.025	—	0.025

TABLE XVII.—*Agglutination and bacteriolysis of bacilli which are of the same type as "I" (Rio-yang I) in fermentative reactions during five days, with the same sera.*

Bacilli tested.	"Miyake."	"Baltic."	"Ota."	"Okabe."	"Toyama."	"Otomasa."
Anti-dysenteric serum (rabbit serum):						
"Port Arthur" ("B" type, non-acid)—						
Agglutination	80	40	—(1-20)	—(1-20)	200	—(1-20)
Bacteriolysis	0.0025	0.025	0.025	—	0.01	0.0005
"Kyoto" ("O" type, acid)—						
Agglutination	40	200	20	—(1-20)	20	
Bacteriolysis	—	—	—	0.05	0.00025	

It is very interesting to compare the relation of the bactericidal reactions between the fifteen types. In Table XVIII, where only six are selected, we can follow the "cross bacteriolysis" between the types. The positive sign running obliquely from each corner above to the other one below and crossing is very striking. From all these observations we are compelled to consider all fifteen of the types as belonging to a single group of the dysentery bacillus.

The fact that these fifteen types of dysenteric bacilli are morphologically and culturally similar and that they show but slight variations in fermentation, agglutination, and bacteriolysis may perhaps be useful in the future in advancing our knowledge of systematic bacteriology, a subject which is at present very obscure.

TABLE XVIII.—*Cross bacteriolysis of six types of dysentery bacilli tested with homologous sera.*

Sera and type.	Non-acid bacilli.			Acid bacilli.		
	"A."	"D."	"F."	"G."	"H."	"O."
Non-acid sera:						
"A"	+	+	—	—	+	+
"D"	+	—	—	+	+	+
"F"	—	—	—	+	+	—
Acid sera:						
"G"	—	+	+	+	+	—
"H"	+	+	+	+	—	+
"O"	+	+	—	—	+	+

SUMMARY AND CONCLUSIONS.

In order that complete observations might be obtained, 74 strains of dysentery bacilli were isolated, collected, and identified from several sources, Japan, Korea, Manchuria, the Philippine Islands, and the Japanese and Baltic fleets (during the Russo-Japanese war). Upon a comparison of all these strains it was found that they could not be distinguished from one another either by their morphological or cultural properties, although minor differences were shown to exist in their fermentative powers and their reaction toward immune serum.

In my experiments I was not satisfied in attempting a differentiation of the different types of bacilli, based on the fermentation and agglutination reactions alone, but in addition their bacteriolytic action toward immune sera was employed.

(1) The results of the study indicate that the great majority of the bacilli which have been isolated during the past few years from cases of acute dysentery (not due to amœbæ) must be considered as being the exciting factor of the disturbance. The organisms which have come into my possession and which have been isolated as the cause of dysentery may be separated into fifteen groups which have fermentative characters distinguishing them one from the other, six not fermenting, and nine fermenting mannite (so called non-acid and acid bacilli.)

(2) The mannite fermenting types are widely scattered over the world and certainly cause characteristic sporadic cases and epidemics of dysentery. The form of the disease caused by them is often severe. On the other hand, the non-fermenting ones often give rise to milder cases of infection (as in Manchuria).

(3) The grouping of the different organisms, according to the differences in their powers of causing fermentation, does not correspond to that which results from differences observed in agglutinative and bacteriolytic action with specific immune sera. The anti-dysenteric rabbit

sera, prepared with so-called non-fermenting bacilli, often agglutinate strains which ferment mannite in the same or in higher dilutions than they do other organisms of the non-fermenting type, and vice versa. The same phenomena can also be confirmed by bacteriolytic tests, the so-called "cross bacteriolysis" usually taking place between bacilli termed both acid and non-acid.

(4) In consideration of all the above facts it seems to me that no reason exists to separate the dysentery bacilli into two distinct groups, the acid and non-acid, as proposed by Lentz, and I can also see no justification in designating any bacillus which causes dysentery as a "pseudo-dysentery bacillus" according to Kruse. We are compelled to consider the fifteen types of dysentery bacilli as constituting a single group.

Finally, I take great pleasure in acknowledging my indebtedness to Professor Kitasato, director of the Government Institute for Infectious Diseases in Tokyo, for many suggestions and kindnesses; I am also under obligations to Dr. Shiga for important suggestions and kind assistance. I desire also to express my obligations to Dr. Strong, director of the Biological Laboratory, Bureau of Science, Manila; Dr. Momose and Dr. Hirano, naval fleet surgeons of the Imperial Japanese Navy, and Dr. Hata and Dr. Oshida, military lieutenant surgeons of the Imperial Japanese Army for courtesies shown by them in sending me materials for this study. My thanks are also due to Dr. Hidaka for assistance in my experiments.

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(17) Gay: The types of *B. dysenteriæ* (Shiga) in relation to bacteriolysis and serum therapy. *Univ. of Penn. Med. Bull.* (1903), XVI, 171.
(18) Wollstein: The dysentery bacillus in a series of cases of infantile diarrhœa. *J. Med. Research* (1903), n. s. V, 11.
(19) Park, Collins, and Goodwin: The dysentery bacillus group and varieties which should be included in it. *Ibid* (1904), n. s. VIII, 553.
(20) Hiss: On fermentative and agglutinative characters of bacilli of the dysentery group. *Ibid* (1904), n. s. VIII, 1.
(21) Lentz: Immunität bei Ruhr. (Kolle u. Wasserman) *Handb. d. path. Mik.* (1904), IV, 894.
(22) Jurgens: Zur Aetiologie der Ruhr. *Deutsche med. Wchnsch.* (1903), XXIX, 841.
(23) Morgenroth: Ueber Ruhruntersuchungen in Korea, etc. *Arch. f. Schiffs und Trop. Hyg.* (1904), VIII, 27.
(24) Ohno: Die Ruhr in Manchuria und Ruhrbacillen typhus, Saikin Gaku Zasshi. *Arch. f. Bakteriol. Jap.* (1905), No. 111, 15.
(25) Momose: Dysenterie in Korea und ihrer Erreger. *Ibid* (1905), 2.
(26) Hara: Prophylaktische Impfung für Ruhr zu Kanagawa und ihre Resultate. *Ibid* (1905), No. 117, 17.
(27) Oshida and Okada: Dysentery in Port Arthur during the siege. *Ibid* (1905), No. 119, 1.
(28) Ohno: Ruhr Epidemie in Tokyo (1904–1905) und ihrer Erreger (Mittheilung über 102 Fällen der nur acuten und ernsten meist letal geendeten Fällen, ihren Symptomen, pathologischen Veränderungen und ihren Erreger). Official *Ibid* (1905), No. 119–121.
(29) Ohno: Epidemic winter diarrhœa in Tokyo. *Tokyo Med. Journ.* (1905), No. 1401, 21.
(30) Yoshida: On the cause of epidemic dysentery. *Ibid* (1899), No. 1038.
(31) Futaki: Ueber den Erreger der epidemischen Dysenterie in Tokyo. *Mitth. d. Jap. Hyg. Gesellsc.* (1905), I, Heft 2.
(32) Nakajo: Ein Fall. d. Laboratorium Dysenterie Infection und seine bacteriologische Untersuchung. *Saikin Gaku Zasshi* (*Jap.*) (1904), No. 112, 23.
(33) Tuchija: Ueber die Typen der Ruhrbacillen. *Ibid.* (1905), No. 114, 13.
(34) Duval: Another member of the dysentery group. *J. Am. Med. Ass.* (1904), XLIII, 381.

A LIST OF PHILIPPINE CULICIDÆ WITH DESCRIPTIONS OF SOME NEW SPECIES.

By Charles S. Banks.

(*From the entomological section, Biological Laboratory, Bureau of Science.*)

The mosquito problem in its relation to human pathology has assumed such gigantic proportions in all parts of the world during the past decade, and species and genera have been found in such large numbers, especially throughout tropical regions, that little wonder attaches to the discovery of a very great number of species within a comparatively brief period in the Philippines. Osten-Sacken, in his "Diptera from the Philippine Islands, brought home by Dr. Carl Semper,"[1] gives:

Culex, several species, damaged.

Megarhina, one male; determined by Walker as *M. immisericors* Wk., although abdomen and legs do not agree with his description. It may be *amboinensis* Dol., although the agreement is not perfect.

Padre Casto Elera[2] gives in addition *Culex pipiens* Linn., as occurring in all parts of the Archipelago and *Corethra manilliensis* Schiner,[3] so that up to the year 1895 the knowledge of just what species of *Culicidæ* occur in the Islands was, to say the least, very indefinite.

The work of the surgeons of the United States Army in various parts of this region brought under their observation many forms with which they were not familiar, and naturally they were led to send them to persons whose knowledge of the group would enable them to identify the species found and give the physicians working here some idea as to the possible prevalence of pathophoric forms. Among those who have done most in the matter of identification of material are Professor F. V. Theobald, Lieutenant-Colonel George M. Giles, I. M. S. (retired), and Miss Clara S. Ludlow, the latter of the laboratory of the Surgeon-General's Office, United States Army, at Washington. They have also described many new species from the Philippines, and it is therefore but natural that in compiling a list of this kind I should have been very dependent upon their publications.

[1] *Berl. Ent. Ztschr.* (1882), **26**, 96.

[2] *Catalogo de toda la Fauna Filipina* (1895), **2**, 490, 491.

[3] *Reise der Novara, Diptera* (1868), 30.

In my own work with mosquitoes I have already been able to collect in hitherto unexplored regions several new species which will be added to the already long list, and I feel sure that others will be discovered in the material now on hand. Aside from the new species and one new genus, forms not indicated by any of the above-mentioned workers as occurring in these Islands have been collected, both in Manila and in the southern provinces, as will be seen by consulting the bibliography given with each species. No claim can be made for completeness of reference in all cases, but I believe that, with few exceptions, the works I have cited give original descriptions. Where such are not given it was due to lack of literature, a feature not always to be avoided in the Antipodes.

In all, 83 species, subspecies, and varieties, including 7 as new species or varieties, are enumerated, embraced in 30 genera in 6 subfamilies. The Entomological Collection contains probably 8 as yet unidentified species, so that the total number will soon be not far from 95 per cent of the number of species found in the United States and, as very little work has been done in higher altitudes, the number of species will, within the next five years, probably be considerably further increased.

An interesting and valuable feature of the work being done on Philippine mosquitoes is the study of their life histories and habits. A great diversity of habits, constant for members of a given species, is to be found among those so far studied.

The form described in this paper as *Stegomyia fasciata persistans* Banks is of very great interest. It was at first supposed to be and identified as *S. fasciata* Fabr., by me, the material in each case having been collected rather than reared. Later breedings developed a form so unlike the descriptions of *S. fasciata* Fabr., that doubt arose as to the previously identified specimens being *S. fasciata* Fabr., although Theobald and Ludlow had both reported *S. fasciata* Fabr., from almost the identical regions where the aberrant form was found. Great care was exercised in mounting, and all specimens collected or bred were subjected to examination, so that not less than 2,000 have passed beneath my lens. Not a single individual was found among the perfect specimens, namely, those in which the mesothorax was not in the least denuded, which did not display ornamentation quite different from the descriptions of *S. fasciata* Fabr. Unfortunately, it has not been possible for me to examine in recent years a specimen of the true *S. fasciata* Fabr., so that I have been obliged to depend upon the very careful descriptions given by Theobald. Because of the character of his descriptions I feel all the more assured in taking the stand that the form found here, while closely approaching the type, differs from it sufficiently to be considered a subspecies, the *constancy of ornamentation* in the Philippine form being also a feature in favor of separating it as distinct. If this subspecies proves to be distinct from *S. fasciata* Fabr., and provided that *S. fasciata* Fabr., can not be demonstrated as being in the Islands, the importance of its

distinctness might be of the greatest value in modifying the fear that yellow fever, if once the germs were introduced here by infected persons, would find a ready foothold.

However, it has already been pointed out by Strong[4] that a menace lies in the future commercial intercourse between the yellow-fever region of endemicity in the Antilles and the Isthmus of Panama and the ports of the East, including Hawaii, Guam, and the Philippines, when the Panama Canal shall have been completed, from the possibility of introducing the infected *Stegomyia fasciata* Fabr., which would then be able to spread the disease by reason of the *Stegomyiæ fasciatæ* Fabr., which were by him supposed to be widely distributed in the Philippines. Unless further search should prove the presence of this species in these Islands, the fear of an epidemic would be unfounded unless *Stegomyia fasciata persistans* Banks should prove to be able to convey yellow fever.

The study of a fine series of bred specimens of *S. scutellaris samarensis* Ludl., in this laboratory, in which occur many beautiful variations, leads me to the belief that there is a very close relationship between *S. scutellaris* Walk., and *S. fasciata* Fabr., and that their true zoögeographical boundary line lies in the vicinity of the Philippines, but a discussion of this will be left for a future paper.

CULICIDÆ.

I. ANOPHELINÆ Theobald.

MYZOMYIA Blanchard (2).

1. Myzomyia funesta Giles.

Anopheles funestus Giles, *Mem. Liverp. Sch. Trop. Med.* (1900), **2**, 50.
——— ——— *Handbook of Gnats* (1900), 162.
——— ——— Theob., *Mono. Culic.* (1901), **1**, 178, Pl. IV, fig. 13.
——— ——— *Handb. of Gnats* (1902), 2d ed., 318.
Myzomyia funesta Giles, Theob., *Mono. Culic.* (1903), **3**, 34, Pl. 2.
——— ——— Theob., *Gen. Insectorum, Culic.* (1905), 7.
——— ——— Ludlow, *Can. Ent.* (1905), **37**, 135.

Central and western Africa, Sudan; Pampanga, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

2. Myzomyia ludlowii Theobald, *Mono. Culic.* (1903), **3**, 42, figs. 19–22.
——— ——— *Gen. Ins., Culic.* (1905), 7.
——— ——— Ludlow, *Can. Ent.* (1905), **37**, 135.

Pampanga, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*); Manila, P. I. (758, 3603, 4022 *Banks*) (2229 *Banks, Schultze*) (2161 *Schultze*) (4554 *P. G. Woolley*).

This mosquito is by far the commonest of the malaria group. It breeds readily in salt water in the vicinity of Manila.

[4] *Fourth Annual Report, U. S. Philippine Commission* (1903), 586 et seq.

3. **Myzomyia mangyana** Banks, sp. nov., *Phil. Journ. Sci.* (1906), **1**, 991.
Mindoro, Rio Baco, Chicago, P. I. (3290 *R. C. McGregor*).

4. Myzomyia rossii Giles.
Anopheles rossii Giles, *J. Trop. Med.* (1899), **2**, 63.
——————————— *Handb. of Gnats* (1900), 149.
——————————— Theob., *Mono. Culic.* (1901), **1**, 154.
——————————— *Handb. of Gnats*, 2d ed. (1902), 311.
Anopheles vagus Dönitz, *Beitr. z. Kennt. der Anophel.* (1902), 80.
Myzomyia rossii Giles, Theob., *Mono. Culic.* (1903), **3**, 45.
——————————— Theob., *Gen. Ins., Culic.* (1905), 7.
Philippine Islands.

5. Myzomyia rossii indefinita Ludl., *Can. Ent.* (1904), **36**, 299.
Pangasinan, Camp Gregg, Bayambang, P. I. (*W. P. Chamberlain*); Guimaras, P. I.

6. Myzomyia thorntonii Ludl., *Can. Ent.* (1904), **36**, 69.
——————————— *Idem.* (1904), **36**, 299.
——————————— Theob., *Gen. Ins., Culic.* (1905), 8.
Samar, Oras, P. I.; Mindanao, Cotabato, P. I. (*J. W. Thornton*).

STETHOMYIA Theobald (4).

7. Stethomyia pallida Ludl., *Can. Ent.* (1905), **37**, 129.
Pampanga, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

PYRETOPHORUS Blanchard (5).

7a. **Pyretophorus freerae** Banks, sp. nov., *Phil. Journ. Sci.* (1906), **1**, 993.
Manila, P. I. (5975 *Banks*).

8. Pyretophorus minimus Theob.
Anopheles minimus Theob., *Mono. Culic.* (1901), **1**, 186, fig. 55.
——————————— Giles, *Handb. of Gnats* (1902), 321.
Pyretophorus minimus Theob., *Gen. Ins., Culic.* (1905), 9.
——————————— Giles, *J. Trop. Med.* (1904), **7**, 365.
Pampanga, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

9. Pyretophorus philippinensis Ludl., *Can. Ent.* (1905), **37**, 135.
Pampanga, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

10. Pyretophorus pitchfordi Giles, *J. Trop. Med.* (1904), **7**, 365.
Pampanga, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).
Giles mentions this mosquito as coming from Uganda also, but I can find no other reference to it in literature.

MYZORHYNCHUS Blanchard (7).

11. Myzorhynchus barbirostris Van der Wulp.
Anopheles barbirostris V. d. W., Leyd., *Mus. Notes* (1884), **6**, 48.
——————————— Theobald, *Mono. Culic.* (1901), **1**, 146, Pl. A.

11. MYZORHYNCHUS BARBIROSTRIS Van der Wulp—Continued.

Myzorhynchus barbirostris V. d. W., Theobald, *Ibid.* (1903), **3**, 86.
——————————————— Ludl., *Can. Ent.* (1905), **37**, 135.

PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*); RIZAL, Manila waterworks, Camp 320, P. I. (5205 *Banks, Schultze*); MANILA, P. I. (5762 *Banks*); FT. MCKINLEY, P. I. (5783 *C. F. Craig*).

12. MYZORHYNCHUS PSEUDOBARBIROSTRIS Ludl., *J. N. Y. Ent. Soc.* (1902), **10**, 127.
——————————————— Theob., *Gen. Ins., Culic.* (1905), 10.
——————————————— *Can. Ent.* (1905), **37**, 135.

PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

13. MYZORHYNCHUS SINENSIS Wied.

Anopheles sinensis Wied., *Aussereurop. Zweifl. Ins.* (1828), 547.
——————————————— Theob., *Mono. Culic.* (1901), **1**, 137, Pl. XXXVII, fig. 146, & Pl. A.
——————————————— Giles, *Handb. of Gnats* (1902), 305.
Myzorhynchus sinensis Wied., Theob., *Mono. Culic.* (1903), **3**, 89.
——————————————— Giles, *J. Trop. Med.* (1904), **7**, 365.
——————————————— Theob., *Gen. Ins., Culic.* (1905), 10.

PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

14. MYZORHYNCHUS VANUS Walker.

Anopheles vanus Walker, *J. Proc. Linn. Soc. Lond.* (1860), **4**, 91.
Anopheles sinensis annularis Theobald, *Mono. Culic.* (1901), **1**, 142.
Myzorhynchus sinensis annularis Theobald, *Ibid.* (1903), **3**, 90.
——————————————— *Gen. Ins., Culic.* (1890), 10.

PANGASINAN, Camp Gregg, Bayambang, P. I. (*W. P. Chamberlain*); MANILA, P. I. (3723, 3800 *R. C. McGregor*) (4553 *P. G. Woolley*).

NYSSORHYNCHUS Blanchard (10).

15. NYSSORHYNCHUS FULIGINOSUS Giles.

Anopheles fuliginosus Giles, *Handb. of Gnats* (1900), 1st ed., 160.
——————— jamesii Liston, *Ind. Med. Gaz.* (1901), 441.
——————— leucopus Dönitz, *Insekten Börse* (1901), **5**, 37.
——————— fuliginosus Giles, Theob., *Mono. Culic.* (1901), **1**, 132, Pl. 1, fig. 3.
——————————————— *Handb. of Gnats* (1902), 2d ed., 298.
Nyssorhynchus fuliginosus Giles, Theob., *Mono. Culic.* (1903), **3**, 93.
——————————————— Ludl., *Can. Ent.* (1905), **37**, 135.

PANGASINAN, Bayambang, P. I. (*W. P. Chamberlain*).

16. NYSSORHYNCHUS PHILIPPINENSIS Ludl.

Anopheles philippinensis Ludl., *J. Am. Med. Assn.* (1902), **39**, 426.
——————————————— *J. N. Y. Ent. Soc.* (1902), **10**, 128.
Nyssorhynchus philippinensis Ludl., Theob., *Gen. Ins., Culic.* (1905), 10.

ABRA, San José, P. I.

CELLIA Theobald.

17. CELLIA KOCHII Dönitz.

Anopheles kochii Dönitz, *Insekten Börse* (1901), **5**, 18.
——————————————— Theob., *Gen. Ins., Culic.* (1905), 11.

Federated Malay States, Sumatra, Java, Philippines.

II. MEGARHININÆ Theobald.

MEGARHINUS Robineau-Desvoidy (1).

18. Megarhinus amboinensis Dolesch., *Nat. Tijdsch. Ned. Indie* (1864), **14**, 381.
——— Osten-Sacken, *Berliner Ent. Ztschr.* (1882), **26**, 96.
——— Elera, *Cat. de toda la Fauna Filip.* (1895), **2**, 490.
——— Giles, *Handb. of Gnats* (1902), 276.
——— Theob., *Gen. Ins., Culic.* (1905), 13.

Osten-Sacken says that *M. immisericors* may be *M. amboinensis* and. Padre Elera gives it as distinct.

19. Megarhinus lewaldii Ludl., *Can. Ent.* (1904), **36**, 233.

Guimaras Island, Salog, P. I. (*L. I. Le Wald*).

III. TOXORHYNCHITINÆ Theobald.

TOXORHYNCHITES Theobald (1).

20. Toxorhynchites immisericors Walk.
Megarhinus immisericors Walk., *J. Proc. Linn. Soc. Lond.* (1860), **4**, 91.
——— regius Thwaites, *Idem.* (1864), **8**, 102.
——— sublifer Doleschall, *Nat. Tijdschr. Ned. Ind.* (1864), **14**, 382.
Megarhina immisericors Walker, *J. Proc. Linn. Soc. Lond.* (1865), **9**, 7.
Megarhinus immisericors or amboinensis, Dol., Osten-Sacken, *Berl. Ent. Ztschr.* (1882), **26**, 96.
Megarhina immisericors Walk., Elera, *Cat. de toda la Fauna Filip.* (1895), **2**, 490.
Megarhinus immisericors Walk., Theob., *Mono. Culic.* (1901), **1**, 225.
——— gilesii Theob., *Idem.* (1901), 227.
——— immisericors or amboinensis, Dol., Giles, *Handb. of Gnats* (1902), 273.

Ceylon, India, Malay Peninsula, and East Indies.

Osten-Sacken says "one ♂ determined by Walker as *M. immisericors* Walk., although abdomen and legs do not agree with his description. It may be *amboinensis* Dol., although the agreement is not perfect." Padre Elera copies the two species mentioned by Osten-Sacken as each being found in the Philippines.

WORCESTERIA Banks (2).

21. Worcesteria grata Banks, *Phil. Journ. Sci.* (1906), **1**, 780.

Negros Occidental, Bago, P. I., Hacienda "Louisiana," Mailum (6071 *Banks*); Cebu, Cebu, P. I. (5726 *McGregor*).

IV. CULICINÆ Theobald.

MUCIDUS Theobald (2).

22. Mucidus mucidus Karsch.

Culex mucidus Karsch., *Ent. Nachr.* (1887), 25.
Mucidus mucidus Karsch., Theob., *Mono. Culic.* (1901), **1**, 272.
——— ——— Giles, *Handb. of Gnats* (1902), 349.
——— ——— Theob., *Gen. Ins., Culic.* (1905), 17.

Swan River, Delagoa Bay, Whydah, western Africa; Manila, P. I. (404 *P. G. Woolley*).

DESVOIDYA Blanchard (5).

23. Desvoidya fusca Theobald.

Desvoidea fusca Theobald, *Mono. Culic.* (1903), **3**, 135, figs. 75, 76.
——— Ludlow, *Can. Ent.* (1905), **37**, 134.
Desvoidya fusca Theobald, *Gen. Ins., Culic.* (1905), 18.

Kuala Lumpur; Pampanga, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

24. Desvoidya fusca joloensis Ludl.

Desvoidea fusca joloensis Ludl., *Can. Ent.* (1904), **36**, 236.

Jolo, Jolo, P. I.

Blanchard in suggesting this generic name[5] gave it as *Desvoidya* in honor of Robineau-Desvoidy.

25. Desvoidya obturbans Walker ? 1861.

Culex obturbans Walker, *Proc. Linn. Soc. Lond.* (1860), **4**, 91.
Culex ventralis Walker, *Ibid.* (1861), **5**, 144.
Armigeres obturbans Theob., *Mono. Culic.* (1901), **1**, 323.
——— ventralis Walk., Giles, *Handb. of Gnats* (1902), 385.
Desvoidea obturbans Walk., Theob., *Mono. Culic.* (1903), **3**, 138.
——— ——— Ludl., *Can. Ent.* (1905), **37**, 134.
Desvoidya obturbans Walk., Theob., *Gen. Ins., Culic.* (1905), 78.

Ceylon, southern and northern India, Malay Peninsula, East Indies, China, Japan, Formosa; Pampanga, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

26. Desvoidya panalectoros Giles.

Stegomyia panalectoros Giles, *J. Trop. Med.* (1904), **7**, 368.
Armigeres panalectros, Theob., *Mono. Culic.* (1901), **2**, 317.
——— Giles., *Handb. of Gnats* (1902), 386.
Desvoidea panalectros Giles, Theob., *Mono. Culic.* (1903), **3**, 139.
Desvoidya panalectros Giles, Theob., *Gen. Ins., Culic.* (1905), 18.

Perak (*Wright*), Calcutta (*Giles*); Pampanga, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

[5] *Comptes Rendus Hebdom. des Seances et Memoires de la Soc. de Biologie de France* (1901), **53**, 1046.

STEGOMYIA Theobald (6).

27. STEGOMYIA AMESII Ludlow.

Stegomyia nivea amesii Ludlow, *J. N. Y. Ent. Soc.* (1903), **11**, 139.
_____ amesii Ludlow, Theob., *Gen. Ins., Culic.* (1905), 19.

28. **Stegomyia aurostriata** Banks, sp. nov., *Phil. Journ. Sci.* (1906), **1**, 996.

29. STEGOMYIA CRASSIPES Van der Wulp.

Culex crassipes Van der Wulp, *Dipt. der Midd. Sumatra* (1892), 9.
Stegomyia crassipes Van der Wulp, Theob., *Mono. Culic.* (1901), **1**, 320, Pl. XXXIV, fig. 134.
_____ _____ Giles, *J. Trop. Med.* (1904), **7**, 367.
_____ _____ Theob., *Gen. Ins., Culic.* (1905), 19.

Thayetmyo, Upper Burmah (*Watson*), Soeroelangoen (*Van der Wulp*); PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

30. STEGOMYIA DESMOTES Giles, *J. Trop. Med.* (1904), **7**, 367.

PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

31. STEGOMYIA FASCIATA Fabr.

Culex fasciatus Fabr., et auctt. in litt.
Stegomyia fasciata Fabr., Theobald, *Mono. Culic.* (1901), **1**, 289, Pl. XIII, figs. 49–50.
_____ Giles, *Handb. of Gnats* (1902), 372.
_____ _____ Ludl., *Can. Ent.* (1905), **37**, 134.

All parts of tropical world. PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

I have never found this species, nor has it been brought to me by any collector. *S. fasciata persistans* Banks, *subsp. nov.*, is the form which I have always obtained in various parts of the Philippines.

32. **Stegomyia fasciata persistans** Banks, subsp. nov., *Phil. Journ. Sci.* (1906), **1**, 995.

MANILA, P. I., ILOILO, P. I., NEGROS OCCIDENTAL, Bago, P. I., Hacienda "Louisiana," and Mailum (5773 *Banks*); FT. MCKINLEY, P. I. (*Craig*) and various collectors elsewhere.

The most abundant day-flying mosquito in this region and a vicious biter.

33. STEGOMYIA GARDNERII Ludlow, *Can. Ent.* (1905), **37**, 99.

MINDORO, Bulacao, P. I. (*F. Gardner*); PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

34. STEGOMYIA LEUCOMERES Giles, *J. Trop. Med.* (1904), **7**, 367.

PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

35. STEGOMYIA PUNCTOLATERALIS Theobald, *The Entomologist* (1903), **36**, 156.
_____ Giles, *J. Trop. Med.* (1904), **7**, 367.
_____ *Gen. Ins., Culic.* (1905), 19.

Queensland; PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

36. STEGOMYIA SCUTELLARIS Walk.

Culex scutellaris Walker, *J. Proc. Linn. Soc. Lond.* (1859), **3**, 77, et auctt. in litt.

Stegomyia scutellaris Walk., Theob., *Mono. Culic.* (1901), **1**, 298, Pl. XIV, fig. 53.

——————————— Giles, *Handb. of Gnats* (1902), 374.

——————————— Ludlow, *Can. Ent.* (1904), **36**, 299.

PANGASINAN, Camp Gregg, Bayambang, P. I. (*W. P. Chamberlain*).

This species, as also the succeeding, is not mentioned in Theobald's Culicidæ in "Genera Insectorum"—for what reason I do not know, unless it was an oversight.

37. STEGOMYIA SCUTELLARIS SAMARENSIS Ludl., *J. N. Y. Ent. Soc.* (1903).

——————————— *Can. Ent.* (1904), **36**, 71.

——————————— *Ibid.* (1905), **37**, 134.

SAMAR, LEYTE, MINDORO, ILOILO, P. I., NEGROS OCCIDENTAL, Bago, P. I., (6007, 6040, 6086, 6088 *Banks*); MANILA, P. I., FT. MCKINLEY, P. I. (*Craig*) and various collectors.

This seems a widespread mosquito in the Philippines and conforms always with Miss Ludlow's description. *S. scutellaris* Walk., has never been found by me nor brought in by collectors. The entomological collection, Bureau of Science, contains some very interesting varieties of this subspecies, all bred from the same lot of eggs and to be described later. It would seem very probable that these species—i. e., *S. scutellaris* and *S. fasciata*—may be very closely related and possibly intergradation may occur. This, however, will be treated at a later date.

38. (?) STEGOMYIA SEXLINEATA Theobald, *Mono. Culic.* (1901), **1**, 308, fig. 94.

——————————— Giles, *Handb. of Gnats* (1902), 377.

——————————— Giles, *J. Trop. Med.* (1904), **7**, 367.

Trinidad, W. I.; PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

Giles is not certain of the identity of the specimen so labeled in his list referred to above. This form was first recorded from the West Indies.

39. STEGOMYIA STRIOCRURA Giles, *J. Trop. Med.* (1904), **7**, 367.

PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

SCUTOMYIA Theobald (8).

40. SCUTOMYIA NIVEA Ludlow.

Stegomyia nivea Ludlow, *N. Y. Ent. Soc.* (1903), **11**, 139.

——————————— *Can. Ent.* (1905), **37**, 134.

Scutomyia nivea Ludl., Theobald, *Gen. Ins., Culic.* (1905), 19.

There appears to be a confusion of this species with *Stegomyia amesii* Ludl., in "Genera Insectorum."

41. SCUTOMYIA NONSCRIPTA SAMARENSIS Ludl., *J. N. Y. Ent. Soc.* (1903), **11**, 138.

——————————— Theob., *Gen. Ins., Culic.* (1905), 19.

PHILIPPINE ISLANDS.

HULECOETOMYIA Theobald (12).

42. HULECOETOMYIA PSEUDOTAENIATA Giles.

Stegomyia pseudotaeniata Giles, *The Entom.* (1901), 192.
——— Theob., *Mono. Culic.* (1901), **1**, 312, fig. 312.
——— *Handb. of Gnats* (1902), 379.
——— Theob., *Gen. Ins., Culic.* (1905), 20.

India; RIZAL, Manila waterworks, Gorge Camp, P. I. (5596 *Banks*).

This is the first record of this species as from the Philippine Islands. I bred the specimens under conditions similar to those mentioned by Giles. It was common in January at the locality cited.

GRABHAMIA Theobald (21).

43. GRABHAMIA SPENCERII Theob.

Culex spencerii Theob., *Mono. Culic.* (1901), **2**, 99.
——— Giles, *Handb. of Gnats* (1902), 431.
Grabhamia spencerii Theob., *Mono. Culic.* (1903), **3**, 250.
——— *Gen. Ins., Culic.* (1905).

Canada, Idaho; PHILIPPINE ISLANDS.

This is evidently quoted as from the Philippines in error in "Genera Insectorum."

CULEX Linnaeus (24).

44. CULEX ANNULIFERUS Ludlow.

——— annulifera Ludlow, *J. N. Y. Ent. Soc.* (1903), **2**, 141.
——— annuliferus Ludlow, *Can. Ent.* (1904), **36**, 72.
——— annulifera Ludlow, *Ibid.* (1904), **36**, 299.

PANGASINAN, Camp Gregg, Bayambang, P. I. (*W. P. Chamberlain*).

45. CULEX CONCOLOR Desvoidy, *Mem. de la Soc. d'Hist. Nat. de Paris* (1825), **4**, 405.
——— Theob., *Mono. Culic.* (1901), **2**, 107, Pl. XXVIII, figs. 109, 110.
——— ——— Giles, *J. Trop. Med.* (1904), **7**, 368.
——— Theob., *Gen. Ins., Culic.* (1905), 29.

PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

Theobald states that the genus relation of this species is still uncertain.

46. CULEX FATIGANS Wiedmann, *Aussereurop. Zweifl. Ins.* (1828), 10.

Culex aestuans Wied., *Ibid.* (1828).
——— purgens Wied., *Ibid.* (1828), 9.
——— pallipes Meigen, *Syst. Beschr., Supp.* (1838).
——— anxifer Coquerel (Bigot), *Ann. Soc. Ent. Fr.* (1859), 112.
Heteronycha dolosa Arribalzaga, *Dipt. Argent.* (1896), 56.
Culex macleayi Skuse, *Proc. Linn. Soc. N. S. Wales* (1896), 1745.
Culex skusei Giles, *Handb. of Gnats* (1900), 292.
——— Theob., *Mono. Culic.* (1901).
——— Giles, *Handb. of Gnats* (1902), 438.
——— Theob., *Mono. Culic.* (1903), **3**, 225.
——— Theob., *Gen. Ins., Culic.* (1905), 28.

46. CULEX FATIGANS Wiedmann—Continued.

Asia, South, Central, and North America, West Indies, Africa, southern Europe, Australia, Oceanic Islands; MANILA, P. I., ILOILO, P. I., CAVITE, P. I., NEGROS OCCIDENTAL, Bago, P. I. (751, 765, 6001, 6005, 6092 *Banks*), and various collectors.

This is one of the commonest night-flying mosquitoes in the Philippines. It hides by day in clothing and clothespresses.

47. CULEX FRAGILIS Ludlow, *J. N. Y. Ent. Soc.* (1903), **11**, 143.

PHILIPPINE ISLANDS.

48. CULEX GELIDUS Theobald, *Mono. Culic.* (1901), **2**, 20, Pl. XXIV, fig. 93.
——————————— Giles, *Handb. of Gnats* (1902).
——————————— Ludlow, *Can. Ent.* (1904), **36**, 299.
——————————— *Gen. Ins., Culic.* (1905), 26.

Southern and central India, Ceylon, Federated Malay States; PANGASINAN, Camp Gregg, Bayambang, P. I. (*W. P. Chamberlain*).

49. CULEX GELIDUS CUNEATUS Theobald, *Mono. Culic.* (1901), **2**, 22, fig. 159.
——————————— Giles, *J. Trop. Med.* (1904), **7**, 368.
——————————— *Gen. Ins., Culic.* (1905), 26.

Southern India, Federated Malay States; PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*); MANILA, P. I. (4398, 4471, 5740, 5760 *Banks*).

A fairly common mosquito flying at twilight and during early evening.

50. (?) CULEX HIRSUTERON Theobald, *Mono. Culic.* (1901), **2**, 98, figs. 196, 197.
Culex hirsuteros Giles, *Handb. of Gnats* (1902), 451.
——————————— Giles, *J. Trop. Med.* (1904), **7**, 368.
——— hirsuteron Theob., *Gen. Ins., Culic.* (1905), 27.

Virginia, Woodstock, U. S. A. (*F. C. Pratt*); PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

Giles says that "this specimen is so badly rubbed that the identification is doubtful," and I doubt its being the insect which Theobald describes from Virginia, U. S. A.

51. CULEX IMPELLENS Walker, *Proc. Linn. Soc. Lond.* (1860), **4**, 91.
——————————— Theob., *Mono. Culic.* (1901), **1**, 362, figs. 122, 123.
——————————— Giles, *Handb. of Gnats* (1902), 405.
——————————— Giles, *J. Trop. Med.* (1904), **7**, 368.

PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

52. CULEX LUTEOLATERALIS Theobald, *Mono. Culic.* (1901), **2**, 71, Pl. XXVIII, fig. 108.
——————————— Giles, *Handb. of Gnats* (1902), 448.
——————————— Giles, *J. Trop. Med.* (1904), **7**, 368.
——————————— *Gen. Ins., Culic.* (1905), 27.

Natal, western and central Africa, Federated Malay States; PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*); MANILA, P. I. (5728 *Banks*).

A fairly abundant mosquito in Manila.

53. Culex microannulatus Theobald, *Mono. Culic.* (1901), **1**, 353, Pl. XVIII, fig. 69.
——— Ludlow, *Can. Ent.* (1904), **36**, 299.
——— *Gen. Ins., Culic.* (1905), 25.

Central and southern India, Federated Malay States; Pangasinan, Camp Gregg, Bayambang, P. I. (*W. P. Chamberlain*); Cavite, Cañacao, P. I. (*E. R. Stitt*); Manila, P. I. (*Banks*).

This mosquito breeds in brackish water near Manila and Cavite.

54. Culex pipiens Linnaeus, *Ins. Suec.* (1890) (1758), S. N. 2886.
——— Elera, *Cat. de toda la Fauna Filip.* (1895), **2**, 490.
——— Theob., *Mono. Culic.* (1901), **2**, 132.
——— Giles, *Handb. of Gnats* (1902), 437.

Europe, Mediterranean islands, United States, Canada, Egypt, Teneriffe, Madeira, Algeria.

Recorded by P. Elera in his catalogue as being found in the Philippine Islands.

55. Culex quasiunivittatus Theobald, *Mono. Culic.* (1901), **2**, 32, fig. 164.
——— Giles, *Handb. of Gnats* (1902), 429.
——— Giles, *J. Trop. Med.* (1904), **7**, 368.
——— *Gen. Ins., Culic.* (1905), 26.

Mashonaland; Pampanga, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

56. **Culex rizali** Banks, sp. nov., *Phil. Journ. Sci.* (1906), **1**, 999.

Negros Occidental, Mt. Siya-Siya, Volcano Canlaon, P. I. (6083 *Banks*).

57. Culex rubithorax Macquart, *Dipt. Exot., Suppl.* (1850), **4**, 9.
——— Theobald, *Mono. Culic.* (1901), **1**, 416.
——— Giles, *Handb. of Gnats* (1902), 412.
——— Theob., *Mono. Culic.* (1903), **3**, 227.
——— Giles, *J. Trop. Med.* (1904), **7**, 368.
——— Theob., *Gen. Ins., Culic.* (1905), 26.

Southern Queensland, Tasmania; Pampanga, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

Theobald says "this is probably only a spotted variety of *C. concolor* Rob.-Desv."

58. Culex tigripes De Grandpre et de Charmay, "*Les Moustiques*" (*Planters Gaz. Press*) (1900).
——— Giles, *Handb. of Gnats* (1902), 407.
——— Giles, *J. Trop. Med.* (1904), **7**, 368.

Mauritius, western Africa; British central Africa, Mashonaland, Natal, Mombasa, Queensland, Uganda, Transvaal, Straits Settlements; Pampanga, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

59. Culex vagans Wiedmann, *Aussereurop. Zweifl. Ins.* (1828), 545.
——— Theob., *Mono. Culic.* (1901), **1**, 411, fig. 146.
——— Giles, *Handb. of Gnats* (1902), 414.
——— Giles, *J. Trop. Med.* (1904), **7**, 368.

Hongkong, Shanghai (*Lindesay*), China (*Wiedemann*); Pampanga, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

TAENIORHYNCHUS Arribalzaga (25).

60. TAENIORHYNCHUS ARGENTEUS Ludlow, *Can. Ent.* (1905), **37**, 98.

PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

61. TAENIORHYNCHUS LINEATOPENNIS Ludlow, *Can. Ent.* (1905), **37**, 133.

PANGASINAN, Bayambang, P. I. (*W. P. Chamberlain*).

62. TAENIORHYNCHUS WHITMOREI Giles, *J. Trop. Med.* (1904), **7**, 367.

PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

MANSONIA Blanchard (26).

63. MANSONIA ANNULIFERA Theobald.

Panoplites annulifera Theob., *Mono. Culic.* (1901), **2**, 183, Pl. XXX, fig. 120, text fig. 224.

———————— Giles, *Handb. of Gnats* (1902), 356.

Mansonia annulifera Ludlow, *Can. Ent.* (1904), **36**, 299; (1905), **37**, 734.

———————— *Gen. Ins., Culic.* (1905), 32.

India, Ceylon, Federated Malay States; PANGASINAN, Bayambang, P. I. (*W. P. Chamberlain*); PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*); MANILA, P. I. (2715, 4214, 5734 *Banks*) (4114 *G. L. Araneta*).

64. MANSONIA ANNULIPES Walker.

Culex annulipes Walker, *Proc. Linn. Soc. Lond.* (1857), **1**, 5.

—— dives Schiner, *Reise der Novara, Diptera* (1868), 31.

—— nero Doleschall, *Nat. Tijdsch. Ned. Ind.* (1864), **14**, 383.

—— annulipes Walker, Theob., *Mono. Culic.* (1901), **2**, 185, fig. 119; (1903), **3**, 275, Pl. XXX.

———————— Theob., *Gen Ins., Culic.* (1905), 32.

Straits Settlements, Java; MINDORO, Rio Baco, P. I. (3289 *R. C. McGregor*).

65. MANSONIA UNIFORMIS Theobald.

Panoplites uniformis Theob., *Mono. Culic.* (1901), **2**, 180, Pl. XXX, fig. 118.

Mansonia africanus Theob., *Mono. Culic.* (1901), **2**, 187.

—— —— australiensis Theob., Giles, *Handb. of Gnats* (1902), 2d ed., 355 (Ms.).

Panoplites uniformis Theob., Giles, *Handb. of Gnats* (1902), 253.

Mansonia uniformis Theob., Ludlow, *Can. Ent.* (1905), **37**, 134.

India, Ceylon, Federated Malay States, central and western Africa; PANGASINAN, Bayambang, P. I. (*W. P. Chamberlain*); MANILA, RIZAL, FT. MCKINLEY (5207 *Banks, Schultze*) (5751 *Mrs. W. R. Banks*) (5770, 5781 *C. F. Craig*) (5842 *G. L. Araneta*).

This is the most abundant of the *Mansoniæ*, being very troublesome at times in Ft. McKinley and Manila.

FINLAYA Theobald (29).

66. **Finlaya aranetana** Banks, sp. nov., *Phil. Journ. Sci.* (1906), **1**, 1001.

NEGROS OCCIDENTAL, Bago, P. I., Hacienda "Louisiana," Mailum (6066, 6085 *Banks*); MANILA, P. I. (5772 *Banks*).

67. FINLAYA FLAVIPENNIS Giles, *J. Trop. Med.* (1904), **7**, 366.

PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

68. FINLAYA MELANOPTERA Giles, *J. Trop. Med.* (1904), **7**, 367.

PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

69. FINLAYA POICILIA Theobald, *Mono. Culic.* (1903), **3**, 283.
_____ Giles, *J. Trop. Med.* (1904), **7**, 366.
_____ *Gen. Ins., Culic.* (1905), 33.

Quoted in error as *F. poialia* Theob., Giles, *J. Trop. Med.* (1904), **7**, 366.

PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*); NEGROS OCCIDENTAL, Bago, P. I., and Mailum at Hacienda "Louisiana" (6008 *Banks*).

ANISOCHELEOMYIA Ludlow (*Incerta sedis*).

70. ANISOCHELEOMYIA ? ALBITARSIS Ludlow, *Can. Ent.* (1905), **37**, 131.

PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

POPEA Ludlow (*Incerta sedis*).

71. POPEA LUTEA Ludlow, *Can. Ent.* (1905), **37**, 96.

PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

REEDOMYIA Ludlow (*Incerta sedis*).

72. REEDOMYIA PAMPANGENSIS Ludlow, *Can Ent.* (1905), **37**, 94.

PAMPANGA, Angeles, P. I. (*E. R. Whitmore*).

VI. AEDEOMYINÆ THEOBALD.

(*Aedinæ* Blanchard.)

AEDEOMYIA Theobald (2).

73. AEDEOMYIA SQUAMMIPENNA Arribalzaga.
Aedes squammipennis Arrib., *El Nat. Arg.* (1878), **1**, 151, 3.
_____ squammipenna Arrib., *Dipt. Arg.* (1891), 62.
Aedeomyia squammipenna Arrib., Theob., *Mono. Culic.* (1901), **2**, 219; (1903), **3**, 307.
_____ squammipennis Giles, *Handb. of Gnats* (1902), 479.
_____ squammipenna Arrib., Theob., *Gen. Ins., Culic.* (1905), 35.

South America, West Indies, Ceylon, India, Federated Malay States, Sudan; PHILIPPINES, MANILA, P. I. (4464, 5758 *Banks*) (4661 *Schultze*) (5208 *P. G. Woolley*).

Found in numbers at Manila.

URANOTAENIA Arribalzaga (4).

74. URANOTAENIA CAERULEOCEPHALA LATERALIS Ludlow, *Can. Ent.* (1905), **37**, 385.

MINDANAO, Cotabato, P. I. (*E. B. Vedder*).

75. **Uranotaenia falcipes** Banks, sp. nov., *Phil. Journ. Sci.* (1906), **1**, 1004.

RIZAL, waterworks, Camp 320, Manila, P. I. (5210 *Banks, Schultze*).

76. URANOTAENIA NITIDOVENTER Giles, *J. Trop. Med.* (1904), **7**, 368.

PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

MIMOMYIA Theobald (5).

77. MIMOMYIA CHAMBERLAIN Ludlow, *Can. Ent.* (1904), **36**, 297.
PANGASINAN, Bayambang, P. I. (*W. P. Chamberlain*).

RUNCHOMYIA Theobald (9).

78. RUNCHOMYIA PHILIPPINENSIS Giles, *J. Trop. Med.* (1904), **7**, 368.
PAMPANGA, Angeles, P. I. (*E. R. Whitmore*).

HODGESIA Theobald (16).

79. HODGESIA SANGUINEA Theobald, *J. Trop. Med.* (1904), **7**, 17.
(?) ——————— Giles, *Ibid.* (1904), **7**, 368.
PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

HEINZMANNIA Ludlow (*Incerta sedis*).

80. HEINZMANNIA SCINTILLANS Ludlow, *Can. Ent.* (1904), **37**, 130.
PAMPANGA, Camp Stotsenberg, Angeles, P. I. (*E. R. Whitmore*).

VIII. CORETHRINÆ.

SAYOMYIA Coquillett (3).

81. SAYOMYIA MANILLIENSIS Schiner.
Corethra manilliensis Schiner, *Reise der Novara, Dipt.* (1868), 30.
——————— maniliensis [sic] Schiner, Theob., *Mono. Culic.* (1901), **2**, 300.
Sayomyia manilliensis Schiner, Theob., *Gen. Ins., Culic.* (1905), 43.

ETORLEPTIOMYIA Theobald (*Incerta sedis*).

82. ETORLEPTIOMYIA LUZONENSIS Ludlow.
Oreillia luzonensis Ludl., *Can. Ent.* (1905), **37**, 101.
Etorleptiomyia luzonensis Ludl., *Can. Ent.* (1906), **38**, 185.
PANGASINAN, Camp Gregg, Bayambang, P. I. (*W. P. Chamberlain*).

ANOPHELINÆ.

MYZOMYIA MANGYANA, sp. nov.

Very small, grey, with golden hairs on thorax and abdomen, and white and grey upright scales and frontal tuft on head; palpi, banded dark-brown and cream; wings spotted dark-cream and brown; legs pale proximally, dark-brown distally.

♀, length 3.5 millimeters, length of wing 2.75 millimeters, length of proboscis 1.75 millimeters; head with upright, black-brown, forked scales on occiput and sides; a patch of cream-white similar ones medially in front; a tuft of cream-white scales projecting over frons and a series of lateral, long bristles curving forward over eyes which are nearly black. The frontal tuft is nearly as long as head; antennæ light-brown, densely pilose-setose, the first segment being darker brown and quite bare; palpi as long as proboscis, dark-brown-scaled to apex of third segment, the scales being semi-erect toward base; a very narrow ring of cream scales

at apex of second segment; fourth and fifth if divided into three equal areas would be cream-scaled in the first or basal and third or apical areas and black-brown-scaled in the second. The apical would be very slightly longer, owing to the tuft of cream scales at tip. Proboscis uniformly dark-brown-scaled, the tip being scaleless, lighter brown, and bristled.

Prothoracic lobes dark-brown, clothed with narrow scales and bristles; mesothorax with broad, grey, median area, bordered by brown and with sparse, golden, curved, hair-like scales; a very much elongated narrow, dark V-mark extends from the prothoracic lobes across grey median area, meeting the apex of an acute triangular brown spot at posterior margin; scutellum simple and sparsely covered with golden hair-scales; metanotum nude, brown; halteres ocher, dark-knobbed.

Legs quite uniform brown; femora lighter basally; tibiæ somewhat swollen apically and truncated; metatarsi and tarsi dark-brown; in some lights the last tarsi of posterior legs appear light-brown and the bases of all tarsal segments are lighter.

Abdomen a uniform dark-brown, with golden hair scales; the basal ventral segments slightly lighter.

Wings cream, spotted with brown as follows: Four prominent large brown spots on costa, beside 2 small basal and 1 at extreme apex on curve of wing. The 4 large ones are: First $1\frac{1}{2}$ times its own length from base of wing and occupying costa, subcosta, and Vein I equally; second, which is largest, $\frac{1}{4}$ its own length from first and occupying costa and subcosta equally, but on Vein I its second quarter is cream, much as in *M. ludlowii* Theob.; third spot its own length from second and occupying costa and Vein I equally; fourth its own length from third and occupying costa and Vein I. The extreme apical tiny spot referred to above is 5 times its own length from costal spot 4, lying between forks of first submarginal cell. Vein II with brown on each side of cross vein, its anterior fork with 2 spots lying below costal spots 3 and 4, respectively; its posterior fork all brown except extreme base and apex; Vein III with brown spot near base and very tiny spot near apex; Vein IV brown from opposite costal spot 1 to near mid cross vein, then cream, then its entire stem brown with base of forking cream; its anterior fork all brown except slight cream at base and apex; its posterior fork with long brown spot in middle; Vein V with small brown spot near base and at its forking; its anterior fork with 3 long, brown spots, the middle one longest; posterior fork cream on basal, brown on apical half, except cream spot at junction with margin. Vein VI with basal half, cream except a small spot at its middle; apical half brown, not reaching margin. Marginal fringe brown-slate with slight cream near base and cream interruptions at forks of Veins IV and V and at apex of Vein III.

♂ of this species unknown.

Mindoro, Rio Baco, Chicago, P. I. (*R. C. McGregor,* Coll.)

Time of capture, 7 May, 1905, in early morning, the majority of the specimens, all of which are females, being gorged with blood.

Type ♀, No. 3290 in Entomological Collection, Bureau of Science, Manila, P. I.

This mosquito bears a strong superficial resemblance to *M. ludlowii* Theob., except that it is very much smaller and there is a very decided difference in the spotting of the wings.

The species is named from a tribe of semi-civilized peoples inhabiting the interior of Mindoro and known as the Mangyanes.

PYRETOPHORUS FREERÆ, sp. nov.

Small, dark-grey, with light-grey, lanceolate thoracic scales, and golden, hair-like abdominal scales; fore and mid legs with banded tarsi; posterior tarsi snow-white; palpi dark-brown, white tipped and narrowly white banded before apex; a few white hairs at middle of palpi; frontal tuft white and nearly as long as head; antennæ pale-brown, with white hairs.

♀, length 3.25 millimeters; length of wing 2.75 millimeters; length of proboscis 1.5 millimeters; dark-grey; head with posterior black and anterior white, upright, forked scales; several incurving, black bristles posterior to eyes on sides of head; frontal tuft of pure-white, recurved bristles, nearly as long as head; eyes black; antennæ pale-brown with whorls of snow-white bristles and fine, silvery pubescence; palpi equal in length to proboscis, densely black scaled, those basally being semi-erect; a small tuft of 3 or 4 silvery scales at middle dorsally; apex broadly silver-tipped with few scattered brown scales; a narrow, silver band before apex, separated from apical white by a broad black area equal in length to white apex; proboscis entirely dark-brown, tipped with golden scales at apex.

Prothoracic lobes dark-grey with few, white, flat scales; mesonotum dark-grey with indistinct, longitudinal, darker areas; median region clothed with very pale-golden, bluntly lanceolate, flat scales; external to this region the notum is comparatively bare; pluræ sparsely white-scaled; metanotum dark-brown, nude; scutellum with white scales on all lobes.

Abdomen dark-brown-grey, clothed with pale-golden hair-like scales more abundant toward posterior extremity; lamellæ with dark-brown, flat, somewhat truncated scales and a few, long, golden bristles; under surface sparsely clad with white hairs.

Halteres white, black-knobbed.

Legs pale to dark, golden-brown, banded and white-tipped; anterior femora, dark basally, much lighter apically, with a dark spot before knee; mid femora light basally, dark apically with an external white spot before knee; and an internal, longitudinal, pale line; posterior femora uniformly dark-brown above and externally, lighter brown-golden internally and basally; all tibiæ uniformly dark-brown above, the mid and posterior ones having an external, paler, longitudinal line for their entire

length; the posterior tibiæ swollen apically and white-tipped; all metatarsi uniformly dark-brown, on the anterior and mid, broadly on the posterior narrowly white at apex; anterior and mid tarsi very dark-brown, the first and second segments, only, white tipped; posterior tarsi snow-white except basal half of first segment which is dark-brown.

Wings yellow and brown spotted as follows: Costa with 7 dark-brown spots, the basal being the shortest, the second, third and fourth successively longer, the fifth twice length of fourth, from which it is removed by a yellow area equal in length to second dark spot; sixth slightly shorter than fifth from which it is separated by a distance equal to that between fourth and fifth; seventh equal in length to fourth and separated from sixth by its own length. Subcosta with spots similar to 4 and 5 of costa, except that the one below 5 has some yellow scales at its beginning; Vein I with spot under 4 of costa, 3 spots under 5, a small one at beginning a large one in middle and a very small one at end, similar to those of *Myzomyia ludlowii* Theob., in position; a spot under 6 and 7 of the costa; Vein II with 3 dark spots on stem, one before and two after supernumerary, a basal and an apical dark spot on its anterior and a basal, a median and an apical spot on its posterior fork; Vein III with a dark spot on each side of mid cross vein and one apically, the portion before mid cross vein, dark scaled, the stem of its forks being yellow basally, the remainder being dark to fork, its anterior and posterior forks each with 2 darks spots; Vein V with a yellow spot at extreme base, then with a dark spot of same length followed by yellow to apex of posterior fork which is dark, its anterior fork having 3 dark spots, two sub-basal and the other apical; Vein VI with 3 dark spots, one sub-basal, a faint one medial and one apical. Cilia, beginning at apex of wings, alternately yellow and brown turning to grey-brown or nearly white near base.

Mid cross vein slightly more than its own length from supernumerary, the posterior cross vein slightly more remote from mid cross vein.

The unscaled portions of wing membrane have a golden sheen which is quite noticeable.

♂ unknown.

Manila, P. I. (*Banks,* collector).

Time of capture 23 October 1906.

Type ♀, No. 5975 in Entomological Collection, Bureau of Science, Manila, P. I.

This striking species is at once distinguishable by its snow-white hind tarsi, its frontal tuft and thoracic scales, from any other Anophelinæ in the vicinity of Manila.

It is dedicated to Mrs. Paul C. Freer through whom I was led to discover the first specimen of this species seen. This specimen escaped, but the second one, from which this description is made, was caught in my house at the light. The type lacks the mid left leg.

CULICINÆ.

STEGOMYIA AUROSTRIATA, sp. nov.

Head with black-brown and white scales, thorax brown with golden stripes, abdomen dark-brown, light, almost white ventrally; legs dark-brown except femora which are pale.

♀, length 5 millimeters; length of wings 3.5 millimeters; length of proboscis 2.5 millimeters; head covered with broad, flat scales, nearly white, pale-yellow around eyes and broadly in the median and occipital regions; a broad, black patch on either side giving appearance of eyes when viewed from above; a few scattered, upright, dull-grey, forked scales above nape; lateral curved bristles project forward over eyes; cheeks and under cephalic surface pale-yellow; antennæ brown, first segment paler, clothed on internal surface with patch of white scales as is also second segment very slightly; clypeus well developed, having a slight median depression, bare, but with slight pruinescense; palpi short, not $\frac{1}{6}$ length of proboscis, 5-jointed, the ultimate being extremely minute and globose; proboscis dark-brown, slightly swollen at apex which is lighter red-brown.

Prothoracic lobes large, covered with a patch of golden, flat scales and a bunch of rather stout bristles; mesothorax with dark-seal-brown, narrow scales and adorned as follows: A very narrow, golden median line, scarcely perceptible anteriorly, extending from anterior to posterior margin where it dilates to cover the area of the usual bare spot; external to this a broad, golden band, curving outward on each side of the anterior half and slightly inward on the posterior half of the dorsal area, the two being parallel posteriorly, and suggesting the lyre-figure of *S. fasciata* Fabr. External to these lines and anterior to base of wing a faintly suggested, longitudinal stripe of golden scales. Pluræ and coxæ with numerous patches of broad, dirty-white scales. Mid lobe of scutellum with median patch of flat, yellow-white scales bordered by dark-brown ones and with 4 large bristles; lateral lobes with few mixed yellow, white and brown scales and 3 large bristles each; metanotum bare, dark-brown; halteres pale-yellow with dark-brown knobs.

Abdomen above, very dark seal-brown, black in certain lights; posterior margins of segments with golden bristles; bases of fifth and sixth with narrow white bands, while all segments except last show triangular white patches laterally at their bases; venter white-scaled, except last two segments, which are black or dark-brown.

Legs mostly dark-brown; anterior femora brown internally except near base which is cream-white internally and externally, with a fine, cream-white line extending to knee-joint; mid femora similarly marked except for pale area externally at base and having fine, cream-white line internally; knees white, posterior femora all cream-white except a longitu-

dinal area of brown internally at apex; all tibiæ dark-seal-brown with red-brown bristles; posterior tibiæ with an external, elongated patch of cream scales its own length from apex; metatarsi all brown, the posterior having an elongated sagittate white mark at underside of base; all tarsi uniformly seal-brown; ungues of fore and mid legs equal and unidentate, those of hind legs simple and equal. All brown areas appear blue-black in certain lights.

Wings with very heavily dark-scaled costa; other veins normal; first submarginal slightly longer and narrower than second posterior, their respective stems equaling the forks in length; posterior cross vein $2\frac{1}{4}$ times its length from mid cross vein, than which it is $\frac{1}{4}$ longer; supernumerary at an angle of about 135° to mid cross vein. Second long vein makes a very sharp angle at its juncture with first so that it is parallel at this part with supernumerary. The incrassation posterior to Vein V (Austen's Vein VI) is very strongly developed, a character not so marked in other *Stegomyiæ* and therefore, with other features, making the position of this species uncertain to me.

Negros Occidental, P. I., Canlaon Volcano, Mt. Siya-Siya, at altitude of 760 meters.

Time of capture, 24 June, 1906.

Type, ♀, No. 6082 in Entomological Collection, Bureau of Science, Manila, P. I.

This interesting mosquito, collected only at a high altitude on a rainy day, attacks very readily, all the specimens obtained having been caught upon the bare legs of the native carriers. No males were taken, although search of the undergrowth was made.

I am at a loss to know whether to place it with the *Stegomyiæ* or to erect for it a new genus, it having a bare clypeus, no banding upon the tarsi, the incrassate vein well developed and quite removed from Vein V, and being brown and gold, while most or all *Stegomyiæ* are black and white, with occasionally some suggestion of yellow or gold. Owing to its cephalic mesonotal and scutellar characters it should surely be placed in *Stegomyiæ,* but then again it has *5-jointed* palpi. I hope that the obtaining of males may help to clear up the doubt if it can be done in no other way. A large number of specimens were obtained, all quite uniform in size and markings.

STEGOMYIA FASCIATA PERSISTANS, subsp. nov.

Black and dark seal-brown with white and golden-white markings, legs banded white, nearly as in *S. fasciata* Fabr.

♀, head with broad, seal-brown scales, a bare, median occipital sulcus bordered laterally by white scales which unite anteriorly and project between eyes, which are bordered by narrow, white scales; a series of brown bristles project inward and forward over eyes; sides of head

with 2 distinct, white, linear patches separated by a brown patch of equal width; eyes, blue-black; clypeus, first antennal segment and apices of palpi with white scales, those on antennæ being interrupted externally; a few black scales on second joint of antennæ; remaining joints dark-brown, and proboscis dull-black. Prothoracic lobes nearly covered with silver-white scales; mesothorax with narrow, seal-brown scales, shaped and arranged as in *S. fasciata* Fabr. Markings as follows: A broad, silver-white band on each side, curving outward, then inward at middle of thorax and extending back to hinder margin as in *S. fasciata* Fabr. External and parallel to this is a bare or scaleless linear area; between the lyre-shaped figure thus formed, a very short, median golden-white line from anterior margin of mesothorax, then two parallel, sub-median lines extending two-thirds of distance to posterior margin, then a short median extending to brown, bare spot, then 2 parallel sub-median short lines at sides of bare spot, extending to posterior margin; a broken line of white in front of wings, sub-parallel with hinder curve of lyre-figure, its beginning on pleura; halteres light-brown, pale-knobbed. Second to sixth abdominal segments blue-black, with basal, transverse, silver-white bands and basal, lateral spots on terga; seventh segment minus basal band but with white lateral spots, apical margins of all segments with golden hairs dorsally and ventrally. Ventrum white scaled, fifth and sixth with some black laterally and apically; seventh all black, pale-bordered apically.

Anterior femora pale-scaled basally, a narrow line of which extends to apex externally; black along superior edge; knees with white spot on each side of articulation; mid femora black externally, with longitudinal, white hair-line through middle nearly to apex; base and internal surface whitish; knees white; exterior face of posterior femora white nearly to apex, except superior edge which is black as is apex; knees white; all tibiæ black with 2 distinct rows of snow-white spines arranged as on legs of certain *Orthoptera;* all metatarsi and first tarsals brown-black with white basal bands; remaining tarsi on fore and mid legs, uniformly brown-black; posterior second tarsi brown-black, basally white banded; third tarsi, white, apically black banded; last tarsi, pure white. Ungues equal, unidentate on all except posterior legs.

Wing veins, densely scaled; posterior cross vein a little more than twice its length from the mid cross vein, the interior angle of which with the supernumerary is about 135°. The venation and lepidotaxis are very similar to *S. fasciata* Fabr., and *S. scutellaris sumarensis* Ludl.

♂ similar in all essential respects to ♀. Antennæ are conspicuously pale banded; palpi slightly longer than proboscis and with 4 white bands, the apical 2 of which are ventral only; the wings less densely scaled; the posterior cross vein slightly farther removed from mid cross vein and somewhat shorter.

The size in both ♂ and ♀ is extremely variable, specimens of the former measuring from 3.5–5 millimeters and of the latter 4–6 millimeters. Wing length ♂ 1.75–2.25 millimeters; ♀ 2–2.75 millimeters.

MANILA, NEGROS OCCIDENTAL, ILOILO, P. I. (*Banks*, Coll.).

Time of flight: This mosquito has been captured in Manila during every month of the year.

Types of ♂ and ♀ No. 5773 in Entomological Collection, Bureau of Science, Manila, P. I.

This mosquito is the most annoying form found in the Islands and like its congener, *S. fasciata* Fabr., is extremely persistent in its attacks, returning repeatedly however many times it may be repulsed. Upon alighting it walks around to find a suitable place to bite, its favorite points of attack being the back of the neck, the ankles, and the back of the hands when one is holding a book. It will in no case alight upon white articles except such as one may be wearing, such as white socks or white clothes that have been recently removed from the person. Its time of attack is from 1 to 3 p. m. and just as night falls, when the daylight is too dim for reading. It appears also to be a forerunner of a storm, as it usually attacks one with great energy just before signs of a shower appear. This mosquito is very wily, usually attacking one from behind, and when struck at, almost invariably flies behind one's chair to renew the attack as soon as everything appears to be quiet. This is not noticeable with any other species of mosquito so far observed in the Philippines.

CULEX LUTEOLATERALIS Theob.

CULEX LUTEOLATERALIS Theob., *Mono. Culic.* (1901), **2**, 71 and Pl. XXVII, fig. 108.
——————— *Gen. Insectorum, Culicidæ:* (1905), 27.
——————— Giles, *J. Trop. Med.* (1904), **7**, 368.

The Philippine specimens of this mosquito differ from the type by the presence in the ♀ of two parallel, submedian, orange-golden striæ on the mesothorax for its entire length. In the ♂ these stripes are from about the middle to the posterior margin only. The palpi are destitute of any orange scales at the base. Mention should also be made of the fine, golden hairs interspersed among the flat scales of the dorsum of the abdomen and the greenish tinge of the cream-yellow basal bands. It could hardly be said of the Philippine specimens that the hind ungues are nearly straight, rather that they are straighter than in most species of *Culex* and with the tip sharply curved.

A notable feature of this species is the difference in appearance of the ♂ and ♀ wing-scaling. In the former the scales both median and lateral are certainly reduced almost to a minimum, there being never more than 8 on Vein VI for instance, and these lie exactly median, end upon end, while the same is true of Veins II, III, IV and V except that there are some 5 or 6 elongated lateral scales at the end of Vein III and

about the same number on each of the forks of Vein IV; those on the forks of II are much more numerous but never more than 20. The subcostal and Vein I are more densely clothed with rather short, truncated laterals on both sides. The most striking difference is seen in Vein VI in the two sexes, that of the ♂ being almost nude while in the ♀ it is clothed to the distal angle or point of union with the margin.

Specimens found here differ also from the type in having greyish-brown antennal plumes in the ♂. A noteworthy feature of the ♂ palpi is the presence of a kind of elbow a little before the middle of the first joint, which gives them the appearance of having been broken and anchylosed. I have seen no reference to this in literature and have noted it on no drawings. It may account for the missing segments of the palpi of this and other species in which I have noticed its presence. I agree with Theobald in his assertion that "the palpi, both ♂ and ♀ are strikingly different in this species and that it may therefore have to go into another genus." I hesitate to call this a variety of *C. luteolateralis* Theob., until I have seen specimens of the original, but the fresh specimens that I have obtained show differences as marked as those between *Stegomyia scutellaris* Walk. and *S. scutellaris samarensis* Ludl.

Length, 4.5 to 5 mm.

MANILA, P. I. (5728 *Banks*).

Time of capture: 25–31 July, 1906.

This mosquito has the habit of constantly moving its hind legs up and down over its back while at rest upon the very low shrubbery and grass where it delights to hide. It settles and bites readily and also bites when confined in vials.

CULEX RIZALI, sp. nov.

Head brown and golden; palpi white-tipped, thorax with thin, golden parallel and looped lines dorsally and white plural patches; abdomen brown and blue-black above, laterally and ventrally brown with white basal bands; legs uniformly brown or blue-black; metatarsi and tarsi basally white or cream banded.

♀, length 5.5 millimeters; legth of wing 4 millimeters; head with narrow, curved, golden scales on median line and extending over frons as a tuft in which are some golden bristles; long, slender, brown, upright forked scales at and before nape; eyes dark-red-brown, bordered by similar scales anteriorly and by almost white, flat ones posteriorly and ventrally; antennæ pilose-setose; segments brown, pale basally; first segment yellow-ochraceous, nude except on inner surface which has minute, broad, brown truncated scales; second segment more robust than any succeeding and with brown scales like first; palpi dark-brown with few white scales at tip and in middle; clypeus dark-brown, glabrous; margin slightly pruinescent. Several long, dark-brown, curved bristles project forward over eyes at sides.

Abdominal segments clothed dorsally with broad, flat, black and yellow scales, first segment with semi-erect black tufts and numerous golden spines laterally and on posterior margin; second, third, fourth, each with 2 sub-median yellow spots and lateral, yellow, longitudinal bands, those on second being wider anteriorly and coalescing along basal margin; fifth segment marked as third and fourth with also a large, white, medial patch; sixth, with 4 yellow spots, a large, white, medial spot and yellow apical margin; seventh with very large, white spot, the yellow one having coalesced with marginal and lateral yellow areas; eighth almost wholly yellow, with traces of black scales and a large, white spot as in preceding. The 3 ultimate segments perceptibly smaller than preceding. Abdominal segments to seventh having a broad, white L-shaped band on ventral and caudal margins of each tergite; ventrally the abdomen is yellow with scattered, black scales and prominent vertical tufts of black scales on fifth, sixth, seventh, with much smaller ones on third and fourth. Apical margins of all abdominal segments with numerous, golden-yellow bristles. Legs strikingly banded black, yellow and white as follows: Anterior femora mostly white with 6 distinct black bands spotted with yellow and 2 indistinct ones interrupted by yellow or white, base black, apex yellow; tibiæ yellow with 2 black bands on basal third and scattering black and white scales; metatarsi all yellow, save for few scattering black scales and 2 black spots near bases; tarsal segments with fuliginous scales except last which is all yellow; mid femora like anterior, except that banding is less distinct; hind tibiæ with 7 black bands interrupted and spotted with yellow and white, apex yellow; mid metatarsi and tarsi exactly as anterior, except for scattering yellow scales on tarsi; posterior femora and tarsi as anterior; metatarsi white basally and apically, median area yellow, with few black scales, first and second tarsal segments black basally, yellow medially and white apically, third, black basally and apically, its mid third yellow with black scales, last tarsi paler yellow than those of anterior and mid legs. Ungues on anterior and mid legs equal, bidentate, basal dentition being small, obtuse; ungues on posterior legs small, equal, simple.

Wings spotted, yellow, white and brown; costa yellow on basal half; first fourth of apical half with heavy, dark-brown band interrupted by 3 yellow spots and extending across wing to Vein VI as a broad, yellow-spotted band showing itself only on veins; distal portion of costa with 5 brown and 5 yellow, alternate, equal spots. Veins IV and V similarly spotted on their forks; Vein II less so. Vein VI yellow with 2 brown spots at distal end before margin; marginal scales alternately brown and fawn. Vein I white scaled before and after broad dark-brown band; Vein IV brown scaled except forks and single yellow spot before fork. Halteres white basally, with yellow and brown knobs.

♂ quite similar in general appearance and color to ♀; antennæ with

dorsal half of whorls golden-brown, ventral half black-brown; palpi black-brown, with 5, more or less distinct, yellow or yellowish-white bands and dense, golden hairs on apical segment, 3-jointed, the second 1½ times as long as third, the first 5 times length of second and with a median joint-like constriction and an apical tumescence. Thorax marked as in ♀. Abdominal segments yellow dorsally, the second to sixth having each a median, hour-glass mark of black scales; seventh with an obcuneate, black spot, eighth banded sub-apically with black; second to seventh tergites marked on margin as in ♀; ventrum, including tufts, as in ♀. Legs exactly similar to those of ♀. Ungues of fore and mid legs unequal; the larger, bidentate, its basal tooth lateral and spine-like; smaller unidentate, nearly straight. Wings similar to those of ♀, but all markings much paler; broad, brown median band extends to Vein II only. Apex of costa pale-yellow, with 4 brown, interrupted spots; mid costa with 6. There is slight variation in the markings on dorsum of abdomen in both sexes, a tendency to confluence being seen in some. Length of ♂ 4.5 millimeters, length of wing 2.75 millimeters.

Negros Occidental, P. I., Bago, at Mailum on Hacienda "Louisiana," 150 m. and on Siya-Siya Peak, Canloan Volcano, 700 m. (*Banks*, Coll.).

Time of capture, 17–24 June, 1906, between buttresses of large lauaan trees (*Anisoptera thurifera* Blume).

Types of ♂ and ♀, No. 6066 in Entomological Collection, Bureau of Science.

This species is affectionately dedicated to my faithful friend, Señor Don Juan A. Araneta, of Bago, Negros Occidental, in recognition of his lively interest in scientific investigation.

The general pattern of *F. aranetana* is quite similar to that of F. *poicilia* Theobald, but the coloring distinguishes it at once, while the abdominal and wing markings in both ♂ and ♀ serve to separate it from *F. flavipennis* Giles, the only other species with which it might, upon first sight, be confused. This species is apparently purely sylvan,[6] individuals being found only in the spaces between the buttresses of large forest trees, resting upon the bark, the color of which they appear to simulate to a certain extent, so that it is very difficult to detect them, unless one smokes them out or searches for them *en profile*—i. e., by glancing along the surface of the buttress.

All attempts at causing them to bite were futile and nothing is known of their life history except that they breed in the water in the axils of banana leaves. They are by no means abundant. They are not active, soon alighting after being disturbed. Their discovery was due to a chance whiff of smoke blowing into their retreat.

[6] Since the above has been written a specimen of the same insect was bred from water in the axils of banana leaves at my house in the city of Manila.

AEDEOMYINÆ.

URANOTAENIA FALCIPES, sp. nov.

Length 2.5 millimeters, length of wing 1.75 millimeters; dark ochraceous with brown scales; the head brown-scaled medially with azure around eyes; a medially interrupted, azure band beginning beneath root of wings on pleuræ, extending around anterior margin of mesothorax and including prothoracic lobes. Thorax much arched, almost globose.

♀, head with flat scales, a dark, greenish-brown triangular occipital area, bordered anteriorly by a broad band of light-cerulean scales, contiguous to eyes. Two curved bristles at side of head, one projecting forward, the other outward and backward; 2 converging near median line and projecting in front of eyes, and 2 on each side between median pair and lateral bristle, curving mediad, these latter springing from middle of cerulean band of scales. Upright forked scales long, slender; their apices deeply excavated and serrate.

Antennæ having first joint nude, ocher, darker internally, first joint $1\frac{1}{2}$ as long as any succeeding, clothed with fine hairs and irregularly placed bristles; succeeding segments with basal whorls of long and intermediate shorter, brown bristles, the apical being densely bristled or hairy to tip. Palpi, single-jointed, very minute, very acute at apex, $\frac{1}{10}$ length of proboscis, which is densely scaled to tip, and somewhat enlarged apically, with minute, strongly curved bristles or setæ on bilabiate portion; clypeus prominent, pear-shaped. Prothoracic lobes densely covered with flat, cerulean scales, prominent. Mesothorax, brown, with brown, hair-like scales and black, sparsely arranged hairs over entire surface; scutellum with flat, black scales on median and lateral lobes and with marginal bristles. Pleuræ pale, unscaled; spiracles prominent; halteres pale with very dark knobs. Metanotum nude, glabrous.

Abdominal segments dorsally with blue-black, flat, closely applied scales; a few lighter ones basally on each segment; apical margins with golden bristles. Extremity of body appears sharply truncated; ventrum slightly paler with median ochraceous golden area and whole of eighth segment pale golden.

Legs with dark-brown and ochraceous scales; fore femora with thin, longitudinal, pale line from base on internal surface; other segments concolorous; mid tibiæ strongly swollen basally, about twice thickness of others, very pale basally and dark apically; other segments same as in fore legs; hind femora similar to fore, except that pale line is wider and external. All tibiæ markedly swollen apically and truncate, with very long, caudate-acuminate scales and bristles at apex. Posterior metatarsus and first tarsal segment brown, remaining tarsal segments pale, almost cream-colored. Ungues simple, strongly falcate, one on each foot being broader bladed than the other.

Wings brown-hyaline; costal margin with dark, spine-like scales; hind margin slightly excised between forks of Vein V; forked cells slightly more than $\frac{1}{3}$ length of their stems; first submarginal very slightly shorter than second posterior cell; their anterior forks in nearly a straight line with stems; veins with narrow, flat, truncated median and characteristic, elongated lateral scales, the latter lighter; Vein I, with lateral scales only on each side, similar in shape to the median of Veins III, IV, and V. Vein VI strongly bowed before junction with margin; posterior cross vein twice as long as and twice its own length removed from mid cross vein. Supernumerary meets mid cross vein at 135°. Posterior cross vein its own length from forking of Vein V.

♂ very similar in appearance to ♀; palpi are of about same size, but more rounded apically; proboscis slightly more swollen; whorls of bristles on antennæ before middle of each segment, but approaching base of segment toward apex of antennæ so that those of apical or fourteenth segment are nearly basal; second segment with indication of a second incomplete whorl. Legs similar in color and structure to those of ♀ except that all tarsi are lighter than those of ♀; fore ungues are equal in length, similar to ♀; mid ungues very unequal, the smaller being almost a mere bristle, the larger much curved; hind ungues like those of ♀. Wings similar to those of ♀ but with fewer scales; both median and lateral posterior cross veins shorter and nearer to mid cross vein. Halteres as in ♀.

Rizal, P. I., near Camp 320, Manila waterworks (*Banks, Schultze*, Coll.).

Time of capture, 22 February, 1906.

Types of ♂ and ♀, No. 5210 in Entomological Collection, Bureau of Science, Manila.

This tiny species is unlike any other described *Uranotaeniæ* in having all its thoracic metallic markings cephalad. The peculiarly placed metallic cerulean band and the pale, tarsal joints of the hind legs easily distinguish it from any other of this genus, while the peculiar falcate ungues in both ♂ and ♀ are worthy of note.

VOL. I JANUARY, 1906 No. 1

12

THE PHILIPPINE JOURNAL OF SCIENCE

EDITED BY

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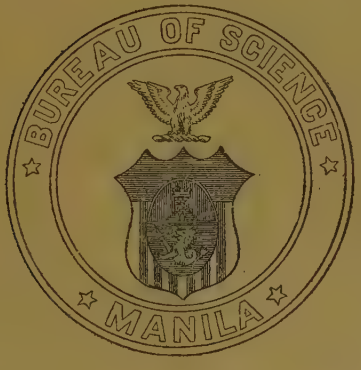
PUBLISHED BY

THE BUREAU OF SCIENCE

OF THE

GOVERNMENT OF THE PHILIPPINE ISLANDS



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BUREAU OF PRINTING
1906

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36512

CONTENTS

Page.

THE PHILIPPINE JOURNAL OF SCIENCE.. 1

FREER, PAUL C. On the Water Relations of the Coconut Palm (Cocos nucifera)—On the Oil Produced from the Nuts—The Factors Entering into the Rancidity of the Oil—The Insects Attacking the Trees. Introduction ... 3

COPELAND, EDWIN BINGHAM. On the Water Relations of the Coconut Palm (Cocos nucifera) ... 6

WALKER, HERBERT S. The Coconut and Its Relation to the Production of Coconut Oil ... 58

WOOLLEY, PAUL G. The Occurrence of Schistosoma Japonicum vel Cattoi in the Philippine Islands ... 83

STRONG, RICHARD P. A Study of Some Tropical Ulcerations of the Skin with Reference to Their Etiology ... 91

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VOL. I FEBRUARY, 1906 No. 2

THE PHILIPPINE JOURNAL OF SCIENCE

031

EDITED BY

PAUL C. FREER, M. D., PH. D.

CO-EDITORS

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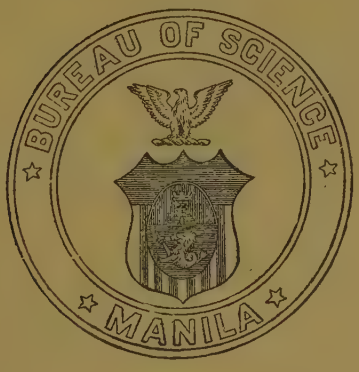

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CONTENTS

Page.

WALKER, HERBERT S. The Keeping Qualities of Coconut Oil and the Causes of Its Rancidity 117

BANKS, CHARLES S. The Principal Insects Attacking the Coconut Palm (Part I) 143

HERZOG, MAXIMILIAN. On Beri-Beri in the Japanese Army during the Late War and on the Kakke coccus of Okata Kokubo 169

STRONG, RICHARD P. Vaccination against Plague 181

CLOVER, A. M. Philippine Wood Oils 191

SMITH, W. D. Orbitoides from the Binangonan Limestone 203

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VOL. I APRIL, 1906 No. 3

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EDITED BY

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CONTENTS

	Page.
BANKS, CHARLES S. The Principal Insects Attacking the Coconut Palm (Part II)------	211
BANKS, CHARLES S. New Philippine Insects ----------	229
BRINKERHOFF, WALTER R., AND TYZZER, E. E. Studies upon Experimental Variola and Vaccinia in Quadrumana (with an Introduction by W. T. COUNCILMAN)-	239

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VOL. I MAY, 1906 No. 4

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CONTENTS

Page.

TYZZER, E. E. The Histology of the Skin Lesions in Varicella 349

WHITFORD, H. N. The Vegetation of the Lamao Forest Reserve 373

BACON, R. F. The Waters of the Crater Lakes of Taal Volcano, with a Note on Some Phenomena of Radio-Activity 433

LEWIS, GILBERT N. Concerning Silver Oxide and Silver Suboxide 439

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VOL. I JUNE, 1906 No. 5

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EDITED BY

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CO-EDITORS

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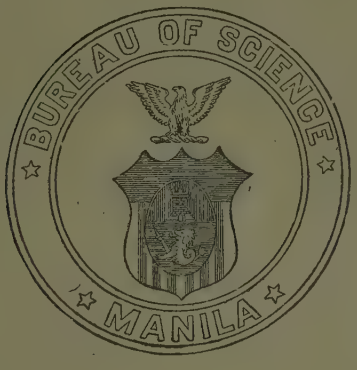

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CONTENTS

Page.

RICHMOND, GEORGE F. Philippine Fibers and Fibrous Substances: Their Suitability for Paper Making 433

KITASATO, SHIBASABURO. Combating Plague in Japan 465

SHIGA, K. Observations on the Epidemiology of Dysentery in Japan 485

STRONG, RICHARD P. A Consideration of Some of Bail's Recent Views in Connection with the Study of Immunity and a Comparison of the Value of Protective Inoculation with Aggressin with that of Vaccination in Plague 501

STITT, E. R. A Study of the Blood in Dengue Fever with Particular Reference to the Differential Count of the Leucocytes in the Diagnosis of the Disease 513

CRAIG, CHARLES F. Observations upon Malaria: Latent Infection in Natives of the Philippine Islands. Intracorpuscular Conjugation 523

WOOLLEY, PAUL G. Tropical Febrile Splenomegaly 533

MUSGRAVE, W. E. Amœbiasis: Its Association with Other Diseases, Its Complications, and Its After Effects 547

REVIEWS 575

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VOL. I JULY, 1906 No. 6

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CONTENTS

	Page.
WOOLLEY, PAUL G. Rinderpest	577
SMITH, W. D. Preliminary Geological Reconnaissance of the Loboo Mountains of Batangas Province	617
WHITFORD, H. N. The Vegetation of the Lamao Forest Reserve, II. (Concluded from p. 431.)	637
BROWN, ROBERT E., S. J. A Catalogue of Philippine Hymenoptera, with Descriptions of New Species	683
McGREGOR, RICHARD C. Notes on Birds Collected in Mindoro and in Small Adjacent Islands	697
REVIEWS	705

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VOL. I SEPTEMBER, 1906 No. 7

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EDITED BY

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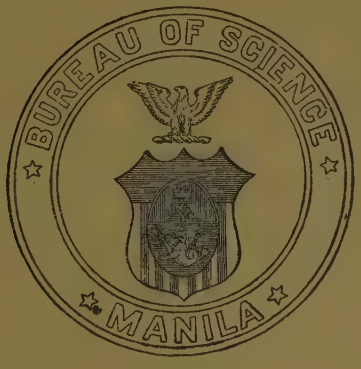

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CONTENTS

	Page.
HERZOG, MAXIMILIAN. Studies in Beriberi	709
McGREGOR, RICHARD C. Notes on Four Birds from Luzon and on a Species of Doubtful Occurrence in the Philippines—Notes on Birds from Apo Island—Notes on a Collection of Birds from Banton—Notes on a Collection of Birds from the Island of Tablas	765
BANKS, CHARLES S. A New Genus and Species of Culicidæ	779
BANKS, CHARLES S. A Change of Name in Coccidæ	787
REVIEWS	789

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CONTENTS

	Page.
WORCESTER, DEAN C. The Non-Christian Tribes of Northern Luzon	791
COX, ALVIN J. Philippine Coals and Their Gas-Producing Power	877
McGREGOR, RICHARD C. Notes on a Collection of Birds from Palawan Island	903

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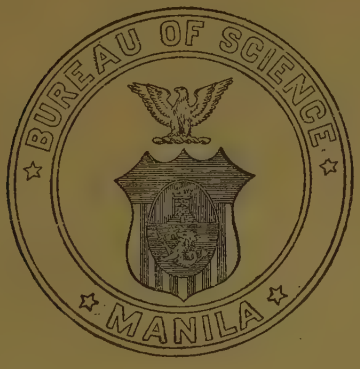

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[1] The first four bulletins in the ornithological series were published by The Ethnological Survey under the title "Bulletins of the Philippine Museum." The other ornithological publications of the Government appeared as publications of the Bureau of Government Laboratories.

CONTENTS

Page.

MUSGRAVE, W. E., and CLEGG, MOSES T. The Cultivation and Pathogenesis of Amœbæ.. 909

OHNO, Y. K. The Types of Bacilli of the Dysentery Group..... 951

BANKS, CHARLES S. A List of Philippine Culicidæ with Descriptions of Some New Species . 977.

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